COMPOSITION AND DIRECTION OF FOREIGN TRADE OF THE ISLAMIC REPUBLIC OF IRAN SINCE 1979

ABSTRACT

Thesis Submitted for the Degree of

Doctor of Philosophy

in

COMMERCE

by

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ALIGARH (INDIA)

1992

ABSTRACT

The Iranian economy has been a dependent economy. The then Government continued assisting the dominanting private sector. Foreign participation and collaboration were also encouraged under the so-called `open door' policy. As a result, the Iranian economy came to be dominated by many foreign firms in the form of subsidiaries, branches and constellation of collaboration agreements (both in agriculture and industry). Yet, Iran by and large remained a one-sector economy, largely dependent upon oil and its increasing exploitation and exports ever since 1908.

Thus, She has been counting upon oil among her natural resources for a period which is no longer than any other nation. Agriculture too has all along been playing an important role in the country. The contribution of oil revenues to capital formation rose from 18 per cent to 60 per cent in 1960s. Between 1973-78, the oil sector contributed on an average nearly 30 per cent to the GDP of the country; services, industry and agriculture, followed the oil sector in receding order. Similarly, in the total exports of the country the contribution of the oil sector has been enormous; touching an annual average of 97 per cent share in the total value of exports during the period 1973-78. Thus, oil revenues provided the opportunities to the Government to launch a series of projects and plans. After the Islamic Revolution in 1979, one of the most important

achievements was the annulment of the right of the oil consortium and prevention of indiscriminate exploitation of the natural reserves in Iran. As per the new policy of the Islamic Government export of oil is done according to the foreign exchange needs of the country; while taking care to conserve oil for future generations.

During 1970s with the increase in oil revenue, the Government instead of paying greater attention to the development of agriculture and industrial base seems to have relied mainly on imports of agricultural and consumer goods. Ultimately, the process adversely affected the Iranian productive activities resulting in very poor technique and languishing units at home. The strengthening of the non-oil productive sector in Iran especially her agriculture, was disregarded by the deposed Shah's regime. One can verify this factor by looking to the growth rates in agriculture during the development plans. The growth rate in agriculture during the Third Development Plan was below the target. It is estimated to be around 25 per cent only. Again, during the Fourth Development Plan the growth rate in agriculture was lower than the target when it stood at 40 per cent. Inspite of the heavy investment to increase the efficiency of agriculture during the Fifth Development Plan, the result achieved added to an increased dependence on food imports. As a consequence, Iran became a net importer of agricultural commodities including grains during 1970s. When imports of foods

and live animals into Iran increased dramaticaly from $ 852 million worth during 1974-75 to $ 1,940 million worth during 1976-77.

The industrial sector has also been the depressed one. Most of the industries prior to Revolution were established without a comprehensive study of the Iranian socio-cultural milieu. Moreover, the industrial planners in Iran could not designate a model indicating a project plan to utilise the local resources. There seemd lack of proper industrial planning, especially after the increase of oil revenues in 1973. No wonder if the huge financial resources from oil and other Governmental revenues were not used to lay a sound foundation for industry and develop a national industry free from dependence. Instead, oil revenues were wasted upon grandoise industrial projects which increased the dependence upon foreign sources and built up powerful monopolies. The factors hindering a rapid industrialization in Iran included :

(i) Under-utilisation of natural resources,

(ii) Low capital formation,

(iii) Low level of Technology,

(iv) Population explosion,

(v) Lack of infra-strucutral facilities,

(vi) Political instability, and

(vii) Social attitudes.

After the Islamic Revolution, the Government assigend top priority to agriculture as a 'central pole' of development to help attain self-sufficiency and to reduce the economy's dependence on the petroleum sector. It may be stated that the gorss neglect of agriculture under the Shah's rule is being reversed with considerable State aid and investment for livestock, gardening and agriculure cooperatives. Rural coopratives numbering over 3,000 and grouped into 180 unions, cover half of the country's farming population. Credits included Rls. 25,000 million and $ 12 million in foreign exchange have been made available for research and training under a reform programme introduced by the Government. During the first post-war Five Year Development Plan (1989-90/93-94) investment in agriculture and natural resources has been estimated at $ 41 billion out of a total of $ 208 billion. It mainly aims at the development of agricultural resources in order to increase the output for strategic products.

Industrialization also entered a new phase aiming at conscious integration of agriculture and industry. The status of the industry was thoroughly reviewed and new policies and plans were formulated by the Government based on the desire to achieve self-sufficiency in every sector of industry in due course of time. Relaince was placed on borrowed technology but only on a selective basis from friendly countries and on reasonable terms.

In order to boost industrial growth, an amount of $ 11 billion was allotted for investments for new industries during first Five Year Development Plan ending in 1994. The investment was designed to boost industrial sectors growth by atleast 8 per cent and to help reduce inflation. Further, in order to achieve the goals of social justice and economic self-sufficiency to become feasible, the Islamic Republic had outlined a Plan for the economic reconstruction. The objectives of the industrial policy and various incentives provided to the industrial sector have not been only to increase output but also to increase competitiveness in domestic and international markets.

Thus, it can be said that inspite of facing difficulties of all sorts, the Islamic Republic of Iran is trying her best to pursue the avowed policy of development with honour and by adhering the 'Neither East nor West' policy.

The foreign trade of Iran in the past reflected the economy's structural weakness. Iranian imports mostly consisted of consumer goods and intermediate goods and that too mostly from the Western bloc, supplying 80 to 85 per cent of the total. After the revolution, Iran's foreign trade policy was modified to provide the country with the essential commodities as far as possible from non-colonial and friendly countries and to stop the import of less- and non-essential products.

The new policy has rightly emphasized at closer economic and political relations between Iran and the Third World countries. Steps were also taken to conduct foreign commercial relations on a more equal and balance footing. Likewise, priority was given to trading with Asian, African and other Non-aligned nations with potentials for economic cooperation in the required areas. Consequently a new trading pattern has emerged. Trade between Iran and the principal OECD suppliers started declining. Instead the Third World countries share of exports rose to 21 per cent of the total during 1983-84 as against 8 per cent before the revolution. Thus, it may be remarked that Islamic Republic of Iran is interested in moving away from the West whereever possible as she strives to diversify commercial relations for avoiding dependence on a few particular trading partners.

Substantial changes were also introduced in the composition of trade. Hereafter, non-primary commodites are also being exported on a considerably large scale. The trade policy has been directed in importing basic and necessary goods, as against consumer items, particularly the luxury goods which were imported in bulk before the revolution. Custom duties on imports of machineries and raw material used in productive industries, stand abolished by the Government to increase the import of essential raw materials. As a result, the imports of raw materials and intermediate goods increased from 54.7 per cent during 1979-80 to 60 per cent during 1988-89.

Keeping in view the findings and conclusions, we have given some suggestions. These include efforts directed to create such an agrarian framework which may assure in the long run a self-sustaining `agriculture-led growth' particularly in provinces like Khuzestan, Ilam and Bakhtaran[1]; provision of certain facilities by the Government to the small scale units for exports of their products; creation of an Export Consortium of Small Scale industries comprising 50-100 small scale units for developing exports; and the adoption of Trade Centres in order to render integrated marketing assistance and promote inter-regional and internatonal trade. An attempt should also be made to use foreign revenues in buy-back or usuance schemes instead of inviting direct foreign investment, for providing help to promote the country's non-oil export sector. Further, utmost importance should be given to the Iranian traditional handicrafts having less number of global competitors. The hand-woven carpets are one of the important products of Iranian industrial home-work accounting for more than 60 per cent of her total production by this sub-sector and more than 40 per cent of the country's total non-oil exports.

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1. This would become possible by making agriculture a viable source of income, employment and supply of raw materials and foodstuffs.

Similarly, efficient publicity in the overseas market is essentially needed. It should be developed on a sustainable and futuristic basis. A new trade policy should ensure regular meetings of Government officials, leading manufacturers, bankers, importers/exporters to acquaint them with the new and latest developments deserving immediate consideration. Lastly, cooperation amongst industrial units within the country, and establishment of a Technology Transfer Fund (TTF) with Advisory Services to finance essential transfer of techonology particularly in capital goods sector, are also needed. The Fund while offering liaison with the exporters/importers of technology, must coordinate and finance the activities of the scientists, technologists and design engineering personnel.

BIOPHYSICAL, BIOCHEMICAL AND HISTOLOGICAL STUDIES ON PERITONEUM

THESIS SUBMITTED
TO
THE ALIGARH MUSLIM UNIVERSITY, ALIGARH
FOR THE DEGREE OF

Doctor of Philosophy

IN

CHEMISTRY

BY

MOHD. SHOAIB

DEPARTMENT OF CHEMISTRY
ALIGARH MUSLIM UNIVERSITY
ALIGARH (INDIA)

1989

D E D I C A T E D

TO

MY FATHER

C E R T I F I C A T E

This is to certify that the thesis for Ph.D entitled **"BIOPHYSICAL, BIOCHEMICAL AND HISTOLOGICAL STUDIES ON PERITIONEUM"** embodied the original work carried out by **Mr. MOHD. SHOAIB** under our supervision. He has fulfilled all the requirements for the degree of **DOCTOR OF PHILOSOPHY** in **CHEMISTRY** of **ALIGARH MUSLIM UNIVERSITY, ALIGARH,** regarding nature and period of the investigational work.

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ACKNOWLEDGEMENT

I wish to acknowledge my deep sense of gratitude to my supervisor Dr. Hasan Arif, Reader, Department of Chemistry, Aligarh Muslim University, Aligarh, for his invaluable suggestions and scholarly guidance in writing this treatise.

I have incurred a debt of gratitude to my co-supervisor Prof. Mohammad Ahmad, Director, Centre of Cardiology and Cardiovascular Research, J.N. Medical College, Aligarh Muslim University, Aligarh, who provided the stimulus for undertaking this problem and during the course of this study he extended invaluable suggestions and advice inspite of his multifarious pre-occupations.

I owe a profound intellectual debt to Prof. Mahdi Hasan, Ex-chairman, Department of Anatomy, J.N. Medical College, Aligarh Muslim University, Aligarh, for his fruitful advice and encouragement during the course of this study.

Thanks are also due to Prof. S.A.A. Zaidi, Chairman, Department of Chemistry, Aligarh Muslim University, Aligarh, for providing requisite facilities.

I am also thankful to Indian Council of Medical Research, New Delhi for providing financial assistance.

I express sincere regards to my mother for her sacrifices and good wishes during the entire period of the study. I also wish to express my thanks to my uncles who have been a great source of inspiration to me in my higher academic pursuit.

I will be failing in my duty if I fail to record my sincere thanks to my research colleagues, friends and well-wishers.

Thanks to Mr. Suhail Ahmad (M/s Micro Services Centre) for word-processing of this thesis.

Last but not the least, thanks are due to Khalid, Aijaz, Adil, Shaukat Ali, Shahabuddin and S. Mujeeb for providing their technical assistance.

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August 10, 1989

CONTENTS

CHAPTER-1

INTRODUCTION AND RATIONALE OF WORK

INTRODUCTION

The present work has been carried out to study certain biochemical, histological and thermodynamical properties of buffalo (Bof. bubalis) peritoneum. The biochemical parameters consisted of estimation of total lipid, cholesterol, phospholipids, triglyceride, free fatty acid, ATPases and trace metal elements viz; Calcium, Magnesium, Iron, Copper, Nickel, Cobalt, Chromium, Zinc, Manganese, Cadmium and Lead. Histological studies were mainly directed to scanning electron microscopic examination of bovine peritoneum. However, routine histological examination of peritoneum membrane stained with Hematoxyline and Eosine (HE) has been performed.

Special emphasis has been laid to the study of thermodynamical properties of peritoneum membrane based on the laws of irreversible thermodynamics by utilizing the different theories developed by Teorell-Meyer and Sievers (TMS) (1,2), Kobatake et al. (3,4,5) and Tasaka et al. (6). Figure-1 has been drawn to show the experimental set up of membrane potential measurement with a detailed analytical explanation in the chapter entitled "Materials and Methods".

An account of the procedure of Materials and Methods, adopted is summarily presented. A piece of peritoneum

membrane of approximately 2 cm in diameter was removed immediately after the experimental animal was slain and put in Ringer's solution to ensure its viability. It was later interposed between two pyrex glass chambers containing the same electrolyte solution with different concentrations. The electrical potential generated across the membrane was recorded and different thermodynamic parameters e.g. transport number, effective fixed charge density and permselectivity of membrane-electrolyte system were evaluated based on the laws of irreversible thermodynamics.

The rationale of this plan was as follows:

(i) Kobatake et al. and Tasaka et al. had developed certain theories of membrane potential based on non-equilibrium thermodynamics and these were found be applicable to synthetic membranes, such as ion exchanger membranes, parchment supported membranes and bilayer lipid membranes etc. artificially prepared in the laboratory. They observed that transport of ions across these artificial membranes were governed by the laws of irreversible thermodynamics. There observations have been confirmed by investigators the world over. Since the ultimate objective of fundamental researches is its application to the services of the

mankind, it becomes natural to examine the applicability of these laws of non-equilibrium thermodynamics to biological systems. Unfortunately, this aspect, however, did not attract the deserved attention of scientists. The reason for this lapse was mainly lack of interaction between biologists and physical chemists. Experimental model for the study of the properties of biomembranes based on the principle of irreversible thermodynamics designed by various investigators consisted of separation of two solutions by a sheet of artificial membrane. Some physical chemists did isolate the egg shell membrane by dissolving the outer shell with weak acid in an attempt to obtain a biological membrane of sufficient area to be able to conduct thermodynamical studies. But the chemical nature of the membrane got altered by treatment with weak acid and the results could not be applied to living tissues. Ussing et al. (7-11) also endeavoured to examine the applicability of the laws of irreversible thermodynamics to the biological system by studies on frog skin. But the frog skin was too thick to be a suitable structure for such studies. Biological chemists, on the other hand concentrated mainly on studies on biochemical, metabolic and histological studies of isolated cell membranes. But the irreversible thermodynamical studies were not possible on isolated cell membranes

because, as for such studies a thin sheet of membrane is required to suit the experimental set up developed by Kobatake et al. to examine the net effect of the acting transport cells and the passive transport through possibly the interstitial tissue.

We, therefore, had no option but to compromise between these two extremes viz., relatively thick frog skin and inappropriate size of isolated cell membrane. We conducted the thermodynamical studies on peritoneal membrane, which is quite a thin structure and is also available in the form of a sheet to suit the experimental set up developed by investigators, reported elsewhere.

(ii) The importance of characterizing the composition of biological membrane and its behaviour are obvious. Such studies will be of great clinical significance. The biological membranes not only form the covering of the various cells, tissues and organs but also govern the movement of nutrients, toxins and other substances across the membrane. Any change in the structure and function of a membrane will adversely affect the integrity of the cell and, ultimately, that of the living organism itself. Unfortunately, the laws of irreversible thermodynamics which

are expected to influence this critical functions of cell membrane could not be undertaken owing to lack of appropriate experimental model as explained above.

Obviously studies on peritoneum can not be transpolated in toto to the function of individual cell membrane and this is the limitation of such studies. But such studies on peritoneal membrane will definitely form a better model than the studies on frog skin for examining the behaviour of biological membrane as compared to artificial membranes.

(iii) Peritoneum is the largest serous membrane in the body. It is composed of fibrous tissues and cells. Apart from protecting the organs in abdominal part of the body, it also influences the movement of fluid and other substances in the peritoneal sac. Since, peritoneum is extensively used in dialysis in renal and non-renal diseases in man, it would be a very important to examine its thermodynamical behaviour.

(iv) The functional behaviour of biological membranes is influenced by its chemical composition and structure. Such studies are specially lacking on bovine peritoneum. Since bovine peritoneum will be a very suitable structure for conducting the thermodynamical studies, it is considered

appropriate to examine light microscopic and electron microscopic structure of bovine peritoneum. We have confined our studies to the examination of the surface of the bovine peritoneum (scanning electron microscopic studies) which will be important for its transport function. For biochemical studies, we have selected the parameters which are most likely to influence the transport function as well.

(v) We have also described the difficulties and limitations of our experimental model, but the results obtained are significant and it is hoped that the findings of this work will definitely form the basis for future researches on this important and interesting field of irreversible thermodynamical studies with respect to biological membranes. A detailed review on these aspects has been presented and the observations have been critically analyzed under 'Results and Discussion' followed by a summary of the findings.

CHAPTER-2

REVIEW OF LITERATURE

2.1 MEMBRANES

Definition

A precise and complete definition of the word "membrane" is difficult to make and any complete definition given to cover all the facets of membrane behaviour will not be exact and any precise statement will be incomplete (12). It is described in simple term "as a phase, usually heterogeneous, acting as a barrier to the flow of molecular and ionic species present in the liquids and/or vapours contacting the two surfaces". The term heterogeneous has been used to indicate the internal physical structure and external physicochemical performance (1-2, 13-17).

A membrane according to the useful definition, is a solid or liquid film or a layer with a thickness which is small compared to its surface. In the case of ion exchanger membranes, a broader definition has come into use. It includes any ion exchange material, irrespective of its geometrical form, which can be used as a separation wall between two solutions. Many common ion exchanger membranes are planar discs about 1 mm in thickness. However, cylindrical plugs (18,19) or single loads glazed into frames are also called membranes. Two different types of ion exchanger membranes are in use. They are often called

"homogenous" and "heterogenous". "Homogenous membranes" are co-herent ion exchanger gels in the shape of discs, ribbons, etc. Their structure is that of usual ion exchanger resins. They are homogenous only in dimensions which are larger as compared to the mesh width of the matrix. "Heterogeneous membranes" consist of colloidal ion exchanger particles embedded in an inert binder (Polystyrene, polyethylene, wax, etc.). Their mechanical stability is superior, but their electrochemical properties such as conductivity and barrier action are not as good as those of homogenous membranes (2Ø). For scientific investigations, homogenous membranes have been preferred even in the past because of their more uniform structure.

Types of the Membranes

The membranes have been classified in two broad groups - The **artificial membranes** and the **biological membranes**. Natural or biological membranes are found in the living organisms, such as, those covers the cell (cell membrane, organelle membrane and myelin membrane) or those covers the viscera (duramater, pericardium, peritoneum etc.). The natural membranes, present in the living organisms, have applied significance because of its various properties.

Therefore, it is quite interesting to investigate the properties of natural membranes in order to understand the factors influencing the structure and functions of biological membranes. The artificial membranes are prepared in the laboratories from various substances, such as, ion exchange resins, inorganic substances and lipids etc. Various other types of membranes have been prepared artificially such as, parchment supported membranes. Cellophane type of membranes are extensively used in refining the brackish water and in other electrolytic processes. Some membranes are also formed by extracting the constituents of biomembranes itself e.g. monolayer and bilayer lipid membranes (BLM). In studying the nature and properties of biological membrane systems it is found that these synthetic membranes are much advantageous. The parchment supported inorganic precipitate membranes, which fall in the category of model membranes, also mimic some of the properties of biological membranes. Plethora of literature on such artificially prepared membranes are available with detailed reviews on them (21-23).

2.1.1. ARTIFICIAL MEMBRANES

Transport phenomena such as permeation and membrane potential across synthetic membranes have received a great

deal of attention during the last few decades. It has also been postulated that the findings of studies on such synthetic membranes may be transpolated to the biological systems. This has further increased the importance of such studies. There are also many biologically important substances such as, aminoacids, aminoacid dervatives and phospholipids which resemble to some extent membranous structure of the living cell membrane. Attention has, therefore, been specially focussed on synthetic membranes prepared from these substances.

Complex membranes have been prepared by Liquori et al. (24-26), Hays (27), De Korosy (28), Lakshminarayanaiah and Siddiqi (29-31), Siddiqi et al. (32-36) and Beg and co-workers (37-4Ø). Several authors (41-45) have investigated membrane potentials and permeation of salts and evaluated the experimental data based on the theory of Theorell-Meyer and Sievers. Kobatake and co-workers (46-49) derived an equation from the data on membrane potentials and fluxes of the salt for the charge density, mobilities and activity coefficients of ions. Demish and Pusch (5Ø) calculated the membrane potential and resistance for binary and ternary electrolyte systems in ion exchanger membranes. A study was carried out by Minoura and Nakagawa (51) to

measure the membrane potentials of poly (α-aminoacid) membranes. Kinoshita et al. (52) made a significant study on the ionic salt permeabilities and membrane potentials of charged polypeptide membranes. Vink (53) also measured membrane potential and diffusion of sodium chloride in cellulosic membranes and interpreted the results, using modified Nernst-Plank equation. Kimura et al. (54) undertook studies on the membrane potentials of carboxy methyle and carboxy ethyl cellulose membranes. Koh and Silverman (55) studied the transport of hydroxide ions in Nafion membranes based on the heterogeneous membrane model carrying narrow capillaries. Beg et al. (39,40) and Siddiqi et al. (56) measured the conductivity for different artificial membranes bathed in different concentrations of alkali metal chlorides and evaluated the thermodynamic parameters of free energy, enthalpy, entropy and energy of activation with the applicability of the theory of absolute reaction rate. Srivastva (57) gave useful report and obtained the permeability of surfactants solutions across synthetic membranes. He also evaluated the thermodynamic parameters of diffusion. Recently, Srivastva et al. (58,59), also studied the permeability properties of different artificial membranes and evaluated the fixed

charge density, apparent anion mobility and transference numbers of counters ions. Singh et al. (60) measured the membrane potentials across ion exchange membrane at different mean concentrations of aquous NaCl and $BaCl_2$ solution, with a view to examine the linear phenomenalogical equation based on the principle of non-equilibrium thermodynamics. The results were computed to know the permselectivity and fixed charge density on the basis of Kobatake's theory. Kenneth Alonso (61) made an investigation about the movement of inorganic salts, amino acid, amino acid derivative and glucose across ion exchange double membrane which served as a model system to examine the phenomenon of controlled and metabolically independent exchange transport. According to them, these types of systems can be compared with the transport to a known cellular system and it will facilitate in designing of drug delivery system. Bassignana and Reiss (62) made an useful study on the ion transport through one of the interfaces (electrical double layer) between an infinitely thin ion exchanger membrane and electrolyte solution. Jensen et al. (63) investigated the properties of symmetrical cellulose acetate membrane by measuring the EMF of concentration cells with membrane as a separator. Nakagaki and Kingi (64) based their studies to determine the

experimental membrane potential and charge density values of cellulose membrane. Beg and Saxena (65) studied the effect of counter ions on the electrolyte transport through manganese ferrocynade membrane. Mallick et al. (66) made an evaluation of the permeability and membrane potential of various electrolytes through parchment supported membranes and it was concluded that at higher concentration selectivity of this membrane decreases, thereby resulting in marked decrease in membrane potential and increase in the membrane permeability values. Toyoshima et al. (47,67) developed a theory of electrical resistance, membrane potential and permeability of charged membranes immersed in a solution of uni-univalent electrolytes. Higuchi and Iijima (68) also based their studies in similar direction to measure the membrane potential and permeability of NaCl in ion exchanger membranes and thus interpreted the results by means of an equation derived from Schloge's theory. Mobility ratio and effective charge density of the membrane was also evaluated by them. Benavente (69) measured the membrane potentials of cellophane membrane, using different concentrations of electrolyte solutions of NaCl, KCl, $MgCl_2$ and $CaCl_2$ and evaluated the charge density of the membrane and transport numbers of counter ions by analyzing the

experimental data on the basis of TMS theory. Benavente and Fernandez (7Ø) also measured membrane potentials across porous membranes and calculated the transport number of cations from diffusion equation based on irreversible thermodynamic processes. Siddiqi et al. (71) worked on parchment supported membranes and emphasized that the effective fixed charge density is the most important parameters governing the transport phenomena in membranes. The charge density and other parameters were also evaluated by applying the different methods viz., TMS, Altug and Hair, and Kobatake et al., based on the thermodynamics of irreversible processes. Kikuchi and Kubota (72) observed the active and selective transport of K^+ and Na^+ in the polyelectrolyte complex membranes and suggested that the affinity of the carrier, and the changes in both chemical and physical properties of these membranes with H^+ concentration, control the selective transport through them. Kontturi (73) made a study on the transport of trace ions to binary electrolyte systems through a porous membranes and postulated a theoretical model based on Nernst-Plank equation. A method for direct determination of effective fixed charge for the species of polydisperse poly-electrolyte across such model porous membranes, based on the

thermodynamic of irreversible processes, was also presented (74). Malik et al. (75) investigated the functional, diffusive and electrochemical properties of inorganic membrane and calculated the different thermodynamic parameters. Dupeyrat (76) carried out a study to obtain the membrane potential of model membrane analogues to biological membranes placed in two aquous solutions of chloride salts. Hernandez et al. (77) measured apparent transport number for ions of homogenous passive membranes separated by binary electrolytes. Peter and Douglas (78) proliferated an idea about the importance and necessity of membrane parameters, diffusion coefficients and fixed charged concentrations to describe the mass transport of water and binary electrolyte across Nafion membrane. Chakravarti et al. (79) evaluated the fixed charge density of cation exchange membranes by applying different methods. Ibanez et al. (8Ø) obtained a modified method based on the Gouy-Chapman double layer potential, applied to nucleopore membrane for the determination of charge densities. The dependence of these charge densities on the concentration of the solutions has been predicted. A theory has also been proposed by Ohshima and Kondo (81) for the potential distribution across charged membrane having thickness and ionizable groups. The

density of membrane fixed charges were determined as a function of potential. The membrane conductance of a microporous membrane was studied at different temperatures, bathed with same uni-univalent electrolyte solutions, at different temperature (82). Siddiqi et al. (83) and Michalov (84) applied the recently developed theories based on the principle of irreversible thermodynamic for evaluation of structural and transport properties of artificial membranes. Kaibara and Kinizuka (85) examined the scope of non-equilibrium thermodynamics in membrane transport. Interionic correlation between permeating ions were quantitatively estimated by applying the membrane permeability theory to the system where the NaCl and $CsCl_2$ solution phases were divided by synthetic cation membrane. Ibanez et al. (86) determined the ionic permeabilities of Na^+ and Cl^- through passive membranes from the membrane potential measurements.Uragami et al. (87,88) studied the active transport of organic ions and amino acids through the artificial membranes under various conditions.

When a membrane separates two electrolyte solution with a common anion but with different cations at the same equivalent concentration, the membrane potential thus generated between the two sides of the charged membrane is

called bi-ionic potential. Mackay and Mears (89), Toyoshima and Nozaki (90) and Tasaka et al. (91) have proposed a theory for the salt concentration depending upon the bi-ionic potential based on the principle of non-equilibrium thermodynamics. Since then, various papers have appeared in literature where authors have applied and tested the validity of these theories to the synthetic membranes. Beg et al. (92) studied on inorganic precipitate membranes and evaluated the effective fixed charge density and compared the theoretical bi-ionic potential with the experimental one. Various other parameters have also been evaluated to know the order of selectivity of the membrane towards cations. Acerrete et al. (93) studied the behaviours of cation exchange membranes in bi-ionic system formed by pairs of alkali ions. It has been concluded that the consequences of the selectivity and membrane potential in bi-ionic and multiionic systems will be useful in understanding the many intricate electrochemical problems of cell physiology. Recently, Tasaka et al. (94) measured bi-ionic potentials across the cation exchange membranes for KCl-NaCl and KCl-LiCl systems. An increase in the membrane potentials was observed with increase in electrolyte concentration.

Phospholipid membranes have recently been the subject of intensive research as a model for studying the structure and functions of biological membranes. Phospholipids bilayers composed of purified phospholipids serves in general as a simplified model for biomembrane. Some of the properties of such artificial membranes possesses striking similarities to those of natural membranes. Among the properties of membrane, transmembrane potential is produced when there is ion concentration gradient across the membrane which is one of the important subjects in understanding the function of biological membranes. Mueller et al. (95,96) prepared the first bimolecular lipid membranes (BLM) which was used as a model by various investigators. Since then, several investigators (97-99) observed that the membrane potentials are produced by the ion concentration gradient across phospholipid membranes. Several other authors (100-103) worked on black lipid membranes, which serves as a model for other membranous studies. Tien (104) studied the thermodynamic properties while Lauger (105) studied the ion transport mechanism, using black lipid membrane by measuring the electrical properties (capacitance, resistance and conductance). Agin (106) reviewed on the negative conductance and electrodiffusion in black lipid membranes. Chock and Titus (107) described the mechanism of ion

transport through biological and artificial membranes and discussed the effect of alkali cation on enzymic activity and Na - dependent transport of polar organic substances. Ohki and Saure (108) measured the surface potential of phosphatidyl-serine monolayers in the presence of Mg^{+2}, Cd^{+2} and Mn^{+2} ion concentrations and determined the way of binding of these divalent ions to the membrane surface. Sandeaux and Brum (109) determined the transmembrane flux of Na^{+} across BLM and John Gutkneght (110) measured the conductance of lipid membrane to reveal the permeability of Cd^{+2} and Tl^{+2} with the use of tracers. Tripathi et al. (111) measured the EMF and current across model membranes made of different types of phospholipids, for chloride salts of sodium, potassium, and calcium at varying concentration and calculated the transport number of ions, permselectivity and ionic fluxes. Finkelstein and Andersen (112) published a review on the permeability characteristics of Gramicidin in channel A with reference to single file aspect of transport in the BLM. The recognition of the reality of channels in biological membranes has been well established and has intensified interest in the mechanisms of transport through them. Zeevi and Margalit (113) discussed the selective transport of Li^{+} across BLM. Nandi et al. (114) performed the electrical conductivity

measurements on BLM in the presence of various chloride salts while Jose (115) measured the surface charge density and potential of isolectin membrane with or without cholesterol and discussed the various occurring phenomena. Impedance and conductance measurements have also been carried out by Ashcroft et al. (116) on the phosphatidylcholine BLM where individual properties of different region in the membranes, have been determined at different KCl concentrations. An attempt was also made to measure the membrane potential and other empirical parameters in liposome (Fernandez (117)). Jones and Nickson (118) measured the electrical resistance and capacitance of BLMs bathed to each side by potassium chloride solutions. Graham et al. (119) described the physical properties of the planar interfaces between the electrolyte and charged lipid membrane. The model used was different from the Gouy-chapman-Debye-Huckel theory for electrolytes. In order to clarify the contribution made to cellular transmembrane potential, the concentration potential of phospholipid BLMs and surface potential of phospholipid monolayers were measured with respect to salt concentration and surface charge densities (12Ø). Lauger (121) discussed the ion transport mechanism (carrier and channel mechanisms) using artificial bilayer lipid membranes. Recently, a review work

has been published where the author (122) has elucidated in detail, the formation and properties of bilayer lipid membranes. A variety of the electrochemical/biosensors based on bilayer lipid membranes have also been described.

2.1.1.1 MEMBRANE POTENTIAL

The applicability of physical chemistry to the biological membranes phenomena is gaining importance because of their properties to affect the transport of material from one side to another. As a consequence, the thrust of the theoretical description has been the interpretation or explanation of transport processes and its measured effect resulting from the pressure differences, activity differences, potential differences and current flow through membranes. A number of theoretical approaches have been given in this respect e.g. Irreversible thermodynamic approach, Activation barrier kinetic approach, Phenomenalogical equation of motion approach (22).

Apart from the various theoretical concepts used in the investigation of membrane, one of the most important approaches in membrane study is application of electrochemical principles. The contribution of electrochemist to the membrane electrochemistry is the

transfer of perspective and wisdom to the new area. There are many sine qua nons in electrochemisty which have occurred through extensive studies of electrolytes and electrolyte-membrane interfaces. Electrochemists have divided the systems into interfacial and bulk processes and expect the effect of dielectric constants, short range forces, high field near interfaces and local potential on the rate of interfacial processes. The presence of space charge at interfaces is a natural consequence of continuity of potential from one phase to another. The presence of space charge mediated effects in membrane system has been anticipated.

The electric and electro-osmotic phenomena exhibited by biological and synthetic membranes have received a great deal of interest in recent past. An extensive review of literature on early development in the field of electrokinetic phenomena in synthetic membranes was given by Sollner (123). Teorell (124) also made significant contribution to the understanding of electrokinetic phenomena, such as the membrane potential. The membrane potential has been widely discussed by several authors (41-45) on the basis of the theory of Teorell (1) Meyers and Sievers (2). However, there have been few attempts to apply

the comprehensive treatment given by Schlogl (125) to calculate the membrane potentials of complex artificial membranes. Recent contribution in this field has been made by Kobatake et al. (3) and Nagasawa et al. (6) with the pronouncement of their membrane potential theories. During the last few decades, a number of investigators (126-128) computed membrane potential in salt solution with various membranes.

The electrical potential arising across the membrane separating two electrolyte solution of different concentrations are usually measured by contributing to a cell of type as depicted in the chart given below:

Reference Electrode	Solution 1	Membrane	Solution 2	Reference Electrode
Electrode potential	Donnan potential		Donnan potential	Electrode potential
		Diffusion potential		

The reference electrode may be reversible electrode, either Ag-AgCl in chloride solution or calomel electrode

connected to the solution via KCl-Agar bridges. In the case of calomel electrode, cell potential gives the membrane potential directly (liquid junction potential being ignored). These types of measurement, are used to characterize the selectivity of the membrane. The magnitude and sign of the potentials depend upon the nature of the membrane and permeating species. If membrane carries no fixed charges then the magnitude of the potential would be like a liquid junction potential, where as, if the membrane have some fixed charges, then the magnitude of the potential would be determined by the concentration of the external electrolyte and its sign by the nature of the fixed charges. The analysis of the membrane potential measurements yields information regarding such properties such as selectivity, effective fixed charge concentration, and ratio of the counter-ion mobility to co-ion mobility within the membrane. To evaluate the fixed charge density of the membrane by the potentiometric method, Teorell (1) Meyers and Sievers (2) gave a method reviewed by Lakshminarayanaiah (22). Kobatake et al. (3) also derived an equation based on the thermodynamics of irreversible process, for electrical potential generated in the solution separated by membrane. An expression for permselectivity of

membrane electrolyte system has also been given by the same (5). Kamo et al. (5) derived another equation to evaluate charged densities of the membranes based on permeation velocity. Toyoshima and Nozaki (9Ø) derived a theoretical equation for the quantitative description of bi-ionic potential and placed them to experimental test. Diffusion of some electrolytes was also studied by several investigators (129). Since, most of the biological membranes are surrounded by electrolytes containing various monovalent and divalent ions, which are bound to the membrane fixed charge sites, the potential distribution across such a membrane having fixed charge layer was studied in terms of ionic concentrations (2,1-1 electrolytes) and ion binding to fixed charge sites (13Ø,131). The introduction of surface charge Layers should be an improvement over the conventional surface potential treatment for biological membrane surface studies. Recently, a theory has been proposed for the potential distribution across such charged membrane with a surface charge layer of some thickness, ionizable groups of dissociation constant (81). The density of membrane fixed charges due to dissociation were determined as a function of potential. It was suggested on the basis of results obtained that the density of the ionizable groups rather

than their total amount played a fundamental role in the electrostatic behaviour of charged membranes.

If we take the fact for granted that the membranes behave as an ion exchange membrane with fixed charges due to the ionogenic groups and obtain the concentration range of these groups within the membrane from the calculation used in the undermentioned theories, we can propose an explanation for transport phenomena operative under specific situations. An electrostatic barrier, which probably depends on ionogenic groups in the membranes, builds up progressively during diffusion of electrolytes through these membranes. Based on this fact it will be possible to decide as to which of the cations or anions diffuse faster if the diffusion of electrolyte occurs across the membrane.

2.1.1.2 THEORIES OF MEMBRANE POTENTIAL

The measurement for the earliest systematic membrane potential were made by many workers on different membrane systems (2-6,69,9Ø,126-129,133-141). Here the potentiometric evaluation of different membrane parameters are described by using different membrane theories. Brief account of these theories can be summarized as below :

Various theories have been proposed from time to time to account for permeability phenomena in membranes. Teorell

(1) and Meyer and Sievers (2) propounded the theory of membrane potential based on the fixed charge concept. Within this theory, it is assumed in essence that the walls of the pores of the membrane carry internally a definite number of potentially dissociable groups, which is an integral part of the membrane structure. The theory propounded by the Teorell, Meyer and Sievers, abbreviated as TMS theory, the membrane potential is due to the sum of two Donnan potential in each membrane solution interface plus a diffusion potential within the membrane. If fixed charges in the membrane is uniformly distributed and, in the case of dilute solutions, activities are substituted by concentrations, so the following final expression is obtained for the membrane potential (Em) (69):

$$Em = -(RT/F)/W \ln (C_1/C_2)\,[(4C2^2 + \bar{X}^2)^{1/2} + \bar{X}] / [(4C_1^2 + \bar{X}^2)^{1/2} + \bar{X}] + U \ln [(4C_1^2 + \bar{X}^2)^{1/2} + WU\bar{X}] / [(4C_2^2 + \bar{X}^2)^{1/2} + WU\bar{X}] \quad \ldots.(1)$$

Here W = +1 or -1, for anionic and cationic membranes, respectively. $\bar{X}$ is the fixed charge density in the membrane.

Besides, the parameter used,

$$U = (D_+ - D_-) / (Z_+D_+ + Z_-D_-)$$

Where D_+ and D_- are diffusion coefficients of cations and anions, Z_+ and Z_- , their valencies respectively. C_1 and C_2 are the concentrations of the solutions separated by the membrane. R is the gas constant and F is the Faraday constant.

Different approximation can be made in equation (1), which depend on fixed charge density value $\bar{X}$ (22) :

(a) if $\bar{X} >> C_2$, then,

$$Em = \pm RT/F \ln (C_2 / C_1), \; C_2 > C_1 \qquad \ldots (2)$$

In this particular case, Nernst's equation is obtained for the membrane potential.

(b) If $\bar{X} << C_2$ then

$$Em = RT/F \; U \ln (C_2 / C_1), \quad C_2 > C_1 \qquad \ldots (3)$$

i.e. a linear relation between Em and ln (C_2/C_1) has been obtained. where, U is proportional to the slope of this straight line.

Since the transport number for a simple salt can be expressed as,

$$t_+ = Z_+D_+/Z_+D_+ - Z_-D_-, \; t_- = Z_-D_-/Z_-D_- - Z_+D_+$$

the factor U can be written as

$$U = (t_+ / Z_+) - (t_-/Z_-) \quad \ldots (4)$$

From equation (3) and (4), the following expressions for membrane potential for each kind of electrolyte result :

For 1:1 electrolyte :

$$Em = RT/F|(2t_+ - 1)|\ln (C_2/C_1) \quad \ldots (5)$$

For 2:1 electrolyte :

$$Em = RT/F \; |(3t_+ / 2) - 1 |\ln (C_2/C_1) \quad \ldots (6)$$

Fro 3:1 electrolyte :

$$Em = RT/F \; | \; (4t_+ / 3) - 1 |\ln C_2/C_1 \quad \ldots (7)$$

The study of membrane potential for different concentrations allows the membrane fixed charge density and the variation of transport number of counter ions within the membrane to be determined as a function of the exterior salt concentration.

Recently, Kobatake et al. (3) expressed the following expression for electric current density (I_c) relative to the frame of reference fixed to the membrane

$$Ic = F\,(l_+ C_+ + l_- C_-)\,(d\phi/dx) - RT\,[l_+ C_+ \, d \ln a_+ / dx) - l_- C_- \,(d \ln a_- / dx)] + F\,(C_+ - C_-)\,Um \qquad \ldots (8)$$

here ϕ is the electrical potential, C_+ and C_- are the concentrations of positive and negative ions in moles per cubic centimeter in solution, a_+ and a_- are the activities of +ve and -ve ions in moles per cubic centimeter of solution, l_+ and l_- are the molar mobilities of +ve and -ve ions defined in terms of mass fixed frame of reference, Um is the velocity of local centre of mass, R,T and F have their usual meanings.

For the evaluation of Um the viscous force acting on 1 cubic centimeter of solution the membrane is represented by $-(1/K)\,Um$, where K is the constant, which is considered to depend on the viscosity of the solution and the structural details of polymer network of which the membrane is composed. The same volume of solution undergoes an electric field which is represented by

$$-F\,(C_+ - C_-)\,(d\phi/dx) \qquad \ldots (9)$$

In the steady state the sum of these two forces is zero, so that

$$Um = -KF\,(C_+ - C_-)\,(d\phi/dx) \qquad \ldots (10)$$

Kobatake et al. (3) for convenience, have considered a membrane which is ionized negatively with charge density Θ (in moles/CC). Then the requirement that the electric neutrality must be realized in any element of the membrane gives the relation.

$$C_+ - C_- = \Theta \qquad \ldots (11)$$

Since, in the system no electric field is applied externally across the membrane, no net charge is transported from one side of the membrane to other. This means that I_c must be zero at a cross section of the membrane.

Substituting equation (11) and (1Ø) into equation (8), keeping I_c equal to zero, and solving for $d\phi/dx$, the following expression is obtained.

$$d\phi/dx = - (RT/F) [l_+ (C_- + \Theta) (d \ln a_+ / dx) - l_-C_- (d\ln a_- / dx)] / [(l_+ + l_-) C_- + l_+ \Theta \quad KF\Theta^2] \qquad \ldots (12)$$

To proceed further, the activities a_+ and a_- must be known as a functions of C_-.

Assumption for a_+ and a_-

The author (3) assumed that

$$a_+ = C_+ \text{ and } a_- = C_- \qquad \ldots (13)$$

or $$\gamma_+ = C_-/(C_- + \theta);\ \gamma_- = 1 \qquad \ldots (14)$$

Where γ_+ and γ_- are the activity coefficients of the +ve and -ve ions in the membrane.

Membrane potential equation

With the assumption of equation (13) and (14), equation (12) becomes :

$$(d\phi / dx) = -(RT/F)\ [\{(l_+ - l_-)\ C_- + l_+\ \theta\ \} / \{(l_+ + l_-)\ C_- + l_+\ \theta + KF\theta^2\}\ C_-]\ (d\ C_- / dx) \qquad \ldots (15)$$

When the bulk solution on the both sides of the membrane are vigorously stirred, no potential gradient is set up in them, so that the desired membrane potential $\Delta\phi$ is obtained by integrating $d\phi / dx$ over the thickness of the membrane. The final expression for membrane potential was given as under:

$$\Delta\phi = -(RT/F)\ [(1/\beta)\ (\ln C_2 / C_1) - (1 + (1/\beta) - 2\alpha)\ X\ \ln (C_2 + \alpha\beta\theta) / (C_1 + \alpha\beta\theta)] \qquad \ldots (16)$$

Where $\alpha = l_+/(l_+ + l_-)$ ... (17a)

and $\beta = 1+(KF\Theta / l_+)$ ... (17b)

The parameters α , β and Θ have been assumed to be independent of salt concentration.

Limiting forms of equation (16)

Kobatake et al. (3) derived two useful limiting forms of equation (16). These are : (a) when the concentration C_2 becomes sufficiently small with γ fixed, equation (16) may be expanded to give the following equation:

$$\Delta\phi_r = 1/\beta \ln \gamma - (\gamma-1)/\alpha\beta\gamma) [1+ (1/\beta) -2\alpha] (C_2/\Theta) + \varnothing [(C_2/\Theta)^2] \quad \ldots (18)$$

Where $|\Delta\phi_r|$ is the absolute value of a reduced membrane potential defined by

$$|\Delta\phi_r| = F \Delta\phi / RT \quad \ldots (19)$$

(b) . It has also been shown experimentally by Kobatake et at. (3) that at fixed γ the inverse of an apparent transference number (tapp) for the co-ion species in a positively charged membrane is proportional to the inverse of the concentration (C_2) in the region of $C_2 >> \Theta$. Where tapp is defined by equation (2Ø)

$$|\Delta\phi_r| = (1- 2\ tapp)\ \ln (C_2/C_1) \qquad \ldots (20)$$

Substituting for $\Delta\phi$ from equation (16) and expanding the resulting expression for 1/tapp in powers of $1/C_2$, gives the equation (21) as,

$$1/tapp = 1/(1-\alpha) + [(1+\beta-2\alpha)(\gamma-1)/2(1-\alpha)^2 \ln\gamma]$$
$$\Theta/C_2 + \emptyset\ [(\Theta/C_2)^2] \qquad \ldots (21)$$

Equation (21) $\emptyset\ [(\Theta/C^2)^2]$ means the second order term and is assumed to be negligibly small compared with the first and second terms on the right hand of this equation in the reign of C2 >> Θ.

Equation (18) indicates that the value of β and a relation between α and Θ may be obtained by evaluating the intercept and initial slope of the plot for $|\Delta\phi_r|$ against C_2, at fixed γ.

Similarly equation (21) indicates that the intercept for a plot of 1/tapp versus $1/C_2$ at fixed γ allows the values of α to be determined. If this value of α inserted in the relation obtained from the initial slope for $|\Delta\phi_r|$ vs C_2, the desired value of Θ can be determined. Once the α and β are known in the manner described above,

the θ may also be evaluated from the initial slope for 1/tapp vs C_2. Provided the membrane potential equation is correct, the two values of θ obtained in this way from opposite limits should agree with one another.

Kobatake and Kamo (4) derived another equation for membrane potential starting with the basic flow equation provided by thermodynamic of irreversible processes and using different set of assumption namely (a) a contribution of mass movement is negligible and (b) small ions do not behave ideally in charged membrane. Their result is,

$$Em = -(RT/F)\left[\ln(C_2/C_1) + (2\alpha - 1)\left\{\ln\left(4C_2^2 + \phi^2 X^2\right)^{1/2} + (2\alpha - 1)\phi X\right\}/\left\{\left(4C_1^2 + \phi^2 X^2\right)^{1/2} + (2\alpha - 1)\phi X\right\} - \ln\left\{\left(4C_2^2 + \phi^2 X^2\right)^{1/2} + \phi X\right\}/\left\{\left(4C_1^2 + \phi^2 X^2\right)^{1/2} + \phi X\right\}\right] \quad \ldots (22)$$

Where ϕ is the characteristic factor of membrane electrolyte pair and represents fraction of counter ions not tightly bound to the membrane skeleton. The ϕX is the thermodynamically effective fixed charge density of membrane and other term have their usual significance. For $\phi = 1$ equation (22) reduces to TMS equation. Since it is difficult to evaluate ϕX by using equation (22) so Kobatake and Kamo (4) proposed simple method using the

equation for diffusion contribution to emf of cell with transport.

$$Em = - (RT/F) (1-2\tau app) \ln (C_2 / C_1) \quad \ldots (23)$$

Where τapp is apparent transference number of co-ions in membrane phases. Comparison of equation (22) and (23) give.

$$\tau app = (1- 2\alpha) /2 \; [\{(4 \xi^2_2+1)^{1/2} + 2\alpha -1\} /\{(4 \xi_1^2+1)^{1/2} + 2\alpha -1\} / \ln \gamma] + [\{(4 \xi_2^2+1)^{1/2} + 1\} / \{4 \xi_1^2+1)^{1/2} + 1\}/ 2 \ln \gamma \quad \ldots (24)$$

Where $\xi = C/\phi X = (C_1 + C_2)/2\phi X$

When the concentration of external salt solution is large as compared to ϕX i.e. $C_1/\phi X = \xi_1 >> 1$ then equation (24) is expanded to give.

$$1/ \tau app = 1/(1-\alpha) + (\gamma-1)/(\gamma \ln \gamma) \alpha /(1- \alpha) (\phi X/C_1) + \emptyset (1/C_1)^2 \quad \ldots (25)$$

This equation (25) indicates that the plot of $1 / \tau app$ vs C_1 at fix γ give a straight line and the values of α and ϕX in the concentrated solution range for a given combination of membrane electrolyte can be determined from the intercept and slope of the line, respectively.

Reduced expression for permselectivity

Both activity coefficients and mobilities of small ions in charged membranes can be expressed according to Kamo et al. (5) by the following equation

$$\gamma_+ = \gamma_+^{\circ} (C_- + \phi X) / (C_- + \phi X), \quad \gamma_- = \gamma_-^{\circ} \qquad \ldots (26)$$

$$u_+ = u_+^{\circ} (C_- + \phi X) / (C_- + \phi X), \quad u_- = u_-^{\circ} \qquad \ldots (27)$$

Here γi ; ui and γi°, ui° (i = +,-) stands for the activity coefficient and mobility of ion species i in the membrane and in the bulk solution, respectively. C_- and X are the concentration of negative ion absorbed in the membrane and stoichiometric concentration of charges fixed in the membrane. According to the convention suggested by Guggenheim (133), γ_+° can be equated with γ_-° for 1:1 electrolyte and they are replaced by mean activity coefficient $\gamma_{\pm}^{\circ}$ of the electrolyte component. In equation (26) and (27) ϕ represents the fraction of counter ion in the unbounded form and ϕX may be referred to as thermodynamically effective fixed charge density of the membrane.

If a charged membrane is immersed in an electrolyte solution of concentration C. Under these condition the

Donnan equilibrium for small ions holds between the membrane phase and the solution, then we get:

$$(\gamma_{\pm}^{o})^2 C^2 = \gamma_+ C_+ \gamma_- C_- \qquad \ldots (28)$$

The mass fixed transference number of ion in the membrane (Tapp) is defined by

$$Tapp = u_- C_- / (u_+ C_+ + u_- C_-) \qquad \ldots (29)$$

Introducing equation (26), (27) and (28) into equation (29) together with the electrical neutrality condition, i.e. $C_+ = C_- + X$, we obtain.

$$Tapp = 1 - \alpha \; [(4\bar{\xi}^2+1)^{1/2} + 1] / [(4\bar{\xi}^2 + 1)^{1/2} + (2\alpha - 1)] \qquad \ldots (30)$$

where $\bar{\xi}$ and α stand for the relative concentration defined by $C / \phi X$ and $u_+^{o}/(u_+^{o} + u_-^{o})$, respectively. On the other hand apparent transference number of the ion in membrane phase (tapp) has been well defined by the equation (20). Kamo et al. (134) has given that the difference between Tapp and tapp was to be less than 2% in wide range of salt concentration when the average concentration (C_1 +

$C_2)/2$ is replaced by C. Equation (3Ø) is applicable even when the concentration on two sides of the membrane are different. Rearrangement of equation (3Ø) leads to

$$1/(4\xi^2 + 1)^{1/2} = [1- Tapp - \alpha]/[\alpha-(2\alpha - 1)(1-Tapp)] \equiv Ps \quad \ldots (31)$$

where Ps is a measure of permselectivity of membrane electrolyte system.

When the external salt concentration C is high enough in comparison with ϕX, i.e. $= C/\phi X >> 1$ the equation of membrane potential, is reduced to

$$\Delta\phi = -(RT/F)(2\alpha-1) \ln (C_2/C_1) \quad \ldots (32)$$

This equation is equal to membrane potential for a system with membrane having no fixed charges. Here, Tapp becomes $(1 - \alpha)$, which in turn, Ps defined by equation (31) reduced to zero. On the other hand, when $C << \phi X$, the equation for membrane potential is simplified to give

$$\Delta\phi = -(RT/F) \ln (C_2/C_1) \quad \ldots (33)$$

which will be the largest potential difference across a charged membrane with a given ratio of concentrations γ =

C_2/C_1. When the membrane potential is in line with equation (33), the membrane may be referred to as perfectly permselective membrane, where Tapp becomes zero and hence Ps tends to unity. So one consider that Ps defined by equation (31) takes a value between zero and unity depending upon the external salt condition for the given system of membrane and an electrolyte pair. Ps can be calculated from the data of membrane potential, while the left hand side of equation (31) is a function of relative concentration $\xi = C / \phi X$ or $(C_1+C_2)/2\ \phi X$. Thus the value of right hand side of equation (31) must be independent of mobilities of ion species involved. Equation (31) implies that Ps should give a straight line of slope unity when Ps is plotted against $(1+4\xi)^{-1/2}$. Kobatake et al. (5) also stated that in the case where a positively charge membrane is concerned, Ps must be defined as $[Tapp-(1-\alpha)]/[1-\alpha-(1-2\alpha)Tapp]$ instead of equation (31).

Tasaka et al. (6) also derived equation (34) for a membrane potential existing across a charged membrane. If a membrane separates two aquous solutions of different concentrations of an electrolyte, at constant temperature and pressure, the fluxes of the water and ions relating to the cell may be expressed by linear equations.

$$-J_o = L_{oo} \text{ grad } \bar{\mu}_o + Lo+ \text{ grad } \bar{\mu}_+ + L_{o-} \text{ grad } \bar{\mu}_- \quad \ldots (34a)$$

$$-J_+ = L_{+o} \text{ grad } \bar{\mu}_o + L_{++} \text{ grad } \bar{\mu}_+ + L_{o+} \text{ grad } \bar{\mu}_- \quad \ldots (34b)$$

$$-J_- = L_{-o} \text{ grad } \bar{\mu}_o + L_{-+} \text{ grad } \bar{\mu}_+ + L_{--} \text{ grad } \bar{\mu}_- \quad \ldots (34c)$$

Where +, - and o refers to cation, anion and water, respectively, Js, to the mass fluxes, Ls, to the phenomenalogical coefficients, and μs, to the chemical potentials. By substituting equation (34a) into (34b) and (34c) we have

$$-J_+ = -(L_{+o}/L_{oo})\, J_o + [L_{++} - (L_{+o}L_{o+}/L_{oo})]x \text{ grad } \bar{\mu}_+ + [L_{+-} - (L_{+o}L_{o-}/L_{oo})] \text{ grad } \bar{\mu}_- \quad \ldots (35a)$$

$$-J_- = (L_{-o}/L_{oo})\, J_o + [L_{-+} - (L_{-+}L_{o+}/L_{oo})]x \text{ grad } \bar{\mu}_+ + [L_{--} - (L_{-o}L_{o-}/L_{oo}) \text{ grad } \bar{\mu}_- \quad \ldots (35b)$$

These equation (35 a) and (35 b) may be approximated as

$$-J_+ = -[(\bar{C}_- + X)/\bar{C}_o]\, J_o + (\bar{C}_- + X)\, \bar{U} \text{ grad } \bar{\mu}_+ \quad \ldots (36a)$$

$$-J_- = -(\bar{C}_-/\bar{C}_o)\, J_o + \bar{C}_-\, \bar{V} \text{ grad } \bar{\mu}_- \quad \ldots (36b)$$

where $\bar{C}_-$ is the effective concentration of counter ions, X is the concentration of fixed charge, and $\bar{U}$ and $\bar{V}$ are the mobilities in the membrane phase.

The total diffusional potential (Em) was considered as the sum of diffusional potential (Ed) inside the membrane and the electrostatic potential difference (Ee) between the membrane surfaces and electrolyte solutions on both sides of the membrane.

From one condition of no current at the steady state, the $J_+ = J_- = Js$ for 1:1 electrolyte, thus we get

$$-F \text{ grad } \psi = -(J_o/\bar{C}_o)\,[X/(\bar{C}_- + X)\,\bar{U} + (\bar{C}_-\bar{V}] + [(\bar{C}_- + X)\,\bar{U} / (\bar{C}_- + X)\,\bar{U} + \bar{C}_-\bar{V}]\,\text{grad}\,\bar{\mu}_+ - [(\bar{C}_-\bar{V})/(\bar{C}_- + X)\,\bar{U} + \bar{C}_-\bar{V}\,\text{grad}\,\bar{\mu}_- \quad \ldots (37)$$

Thus the diffusion potential Ed was obtained by integrating equation (37) for diffusion from one side of the membrane to the other, where as electrostatic potential difference was calculated from Donnan's theory (142)

$$Em = Ed + Ee \quad \ldots (38a)$$

$$\text{Where } -Ed = \int_1^2 J_o X/[F\bar{C}_o(\bar{C}_- + X)\bar{U} + \bar{C}_-\bar{V}]\,dx + (RT/F)\int_1^2 (\bar{C}_- + X)\bar{U} / (\bar{C}_- + X)\,\bar{U} + \bar{C}_-\bar{V}]\,d \ln \bar{a}_+ - RT/F \int_1^2 [\bar{C}_-\bar{V}/ (\bar{C}_- + X)\,\bar{U} + \bar{C}_-\bar{V}]\,d \ln \bar{a}_- \quad \ldots (38b)$$

$$\text{and } -Ee = -(RT/F) \ln (\bar{a}_1 / \bar{a}_2)/(\bar{a}_1 / \bar{a}_2) \quad \ldots (38c)$$

Where a_1 and a_2 are the activities of electrolytes on two sides of the membrane, over bar refers to phenomena in membrane phase, and Jo is the flow of electrolyte in the absence of electric field. By integrating equation (38) and putting the limit of high electrolyte concentrations across the membrane, the following equation for membrane potential is obtained:

$$-Em = (RT/F)\ (X/2)(\gamma-1)/\gamma\ \ 1/C_1 + (RT/F)\ (\bar{U}-\bar{V})/(\bar{U}+\bar{V})\ [(1-XJo)/ RT\ \bar{C}_o\ (\bar{U}-\bar{V})K]\ /\ [(1-X\ Jo)\ /\ 2\ RT\ \bar{C}o\ V\ K\ ]\ x\ \ ln\ \gamma\ + RT/F\ (X/UV)\ \ (Jo/RT\bar{C}oK)^2\ [1-XJo\ \ (U+V)/4\ RT\bar{C}o\ \ UVK/1- XJo/2RT\bar{C}oVK]^2\ x\ [(\gamma-1)\ C_1]^{-1} \qquad \ldots\ (39)$$

At high concentration equation (39) was put in the following approximate form:

$$-Em = (RT/F)\ \gamma/(\gamma-1)\ (\phi X/2)1/C_1 \qquad \ldots\ (4Ø)$$

Equation (4Ø) predicts a relationship between Em and $1/C_1$ from which charge density ϕX can be evaluated.

2.1.2 BIOLOGICAL MEMBRANES

The fundamental unit of biological activity is the living cell and the membranes are conspicuous feature of cell structure, to which it serves not only as a barrier separating aquous compartments with different solutes but also as a structural base to which enzymes and transport systems are firmly bound (132). As a result of the studies carried by cytologists, biochemists and histochemists, it has now become evident that cellular morphology is indispensable for the operation of living activities.

Among the biomembranes, the simplest membrane system is myelin and a second class membrane is plasma membrane, made up of proteins and lipids and has many enzymatic and transport functions (143). There are also a groups of membranes having specific functions, such as, the plasma membrane of bacterial cells and inner membrane of mitochondria. Natural membrane structure is related to structure and properties of lipids and proteins. Rouser (144) gave a detailed quantitative and molecular composition of a variety of erythrocytes, mitochondria, brush border fragments, retinal rod's outer sacs and plasma membranes of adaptable mycoplasma group of organcelles. Biological membranes are composed of mainly lipids, proteins, and carbohydrates in variable proportions. sugar residues are

attached to either lipid or protein components or both. Several models have been proposed by workers (142,145-149) to describe the molecular organization of these constituents in biomembranes, starting from unit membrane theory to "**Fluid Mosaic Model**". Organization of complex structure of biological membranes are related to their divergent and highly specialized functions. In the metabolism of living cells, the role of individual constituents in the organization of structure and function of membranes and membranes organelles are of prime importance in membrane biology.

2.1.2.1 COMPOSITIONS OF BIOMEMBRANES

Most of the biological membranes contain about 4Ø percent of lipid and 6Ø percent protein, but there is also a considerable variations. At one extreme the inner mitochondrial membrane contains only 2Ø-25% lipid, and at the other, the myelin membranes surrounding the certain nerves may contain upto 75% of lipid. The membrane protein can be classified into two categories. The extrinsic or peripheral proteins are loosely attached to the membrane surface and can be easily removed in soluble form by mild extraction procedure. The intrinsic or integral proteins,

which make up 70% or more of the total membrane protein, are very tightly bound and can be removed only by drastic treatment (149). The best information available in this field indicates that proteins of any one type of membranes are grossly heterogeneous. For example, mammalian cell membranes contain a large number of proteins of different molecular weight ranging from less than 15000 to over 100,000. For any given type of membrane, the distribution of protein is quite reproducible, but it differs from membrane to membrane.

The lipids of biomembrane are largely polar in nature, phosphoglycerides predominates, with much smaller amounts of sphingo-lipids and cholesterol. In fact, all the polar lipids of the cells are localized in their membranes. Endoplasmic reticulum and organelle membranes contain relatively little cholesterol or triacylglycerol, where as, plasma membrane of animal cells contains much cholesterol, both free and esterified. Among the phospholipids the natural membrane usually contains high proportion of phosphatidylcholine phosphatidylethamolamine, lysophosphatidylcholine, lysophosphatidylethanolamine, sphingomyelin, phosphatidic acid and cardiolipin. All occupy interfacial space in the membrane but do not

contribute to surface of fixed ions and give rise to electrostatic field extending into the surrounding electrolytes. The magnitude of the potential at a plane of fixed ionic groups can be given by Gouy-Chapman-Equation, analogue to donnan potential. This potential arises because the counter ions to the plane of fixed charge are mobile and their concentration close to the plane, will be always less than that of fixed ions (150).

The other group constituting the membrane are glycoproteins, glycolipids and proteoglycans. The glycoproteins which together with glycolipids constitutes the class of glycoconjugates, results from the co-valent association of carbohydrate moities with proteins. Most proteins are glycosylated, the glycoproteins being widely distributed in animals, plants, micro-organisms and viruses. It has been now well established that glycans perform important biological roles particularly in intracellular recognition and adhesion. It is also well known that glycan structure of cell membrane glycoproteins is profound altered in cancer cells. This molecular mechanism may be related to the appearance of cell surface neoantigens (151). The glycoproteins are associated with the membrane particles as in erythrocytes membrane (152). The two main types of

glycoproteins were found in the membranes of rat liver and kidney cells (153), hella cells (154) and mouse liver cells (155).

Glycolipids are also distributed through out the cells. the glyco-components are oligosaccharide chains, frequently branched and can carry the non carbohydrate constituents usually acetyle or sulphate groups. This is the carbohydrate structures, which confers the biological specificity to a particular glycolipid. Glycerol based glycolipids are common in plants. The ceramide family are found in animal tissues. The neutral glycosphingolipids are important families of sulphoglycosphingolipids and glycosphingolipids which contains sialic acid residue called gangliosides. This animal glycolipids are vital for blood group typing, antigenicity as well as in many metabolic disorders (156).

Sialic acid, a component of glycoproteins and glycolipids, has been found to vary greatly in a number of pathological conditions. It has been reported that it accounts for 95% of the negative charges on the membrane surface erythrocytes (157). Glick et al. (158) carried out an investigation to determine the function of glycoproteins

and glycolipids on cell surface and examined the carbohydrate composition of intact mouse fibroblasts and the whole surface membranes.

Proteoglycans constitutes a variety of polysaccharide components generally consist of repeating disaccharide subunits, of the same or different types. There are five major classes of glycosaminoglycans found in connective tissues, the chondriotin sulphates, keratan sulphates, dermatan sulphates, heparan sulphates and hyaluronic acid. Proteoglycans help to maintain tissue salts and water balance by their capacity to retain large volumes of water within their molecule. Many of the physiological roles of proteoglycans are rely upon the space filling properties. Therefore, proteoglycans exhibit one of the most exquisite structure-function relationships in membranology due to the complex and elegant special and chemical arrangements of molecular component in biopolymers (159).

The major proteins present in the connective tissue are collagen, elastin and proteoglycans. About one third of the body total protein in collagen, of this 50% is present in bone, 40% in skin and 10% in other internal and external organs. Collagen molecules consist of 3 α-chains coiled into triple helix with a rod like structure. The

characteristic feature of collagen is high concentration of proline and hydroxy proline residues. Hydroxy proline represents 1Ø - 15% of the total amino acid residues in collagen. Collagen is a glycoprotein containing galactosyl and glucogalactosyl residue. The carbohydrate unit is attached via an O - glycosidic bone through the hydroxylysine. The tropocollagen molecules are stacked in staggered manner to form the typical fibriller structure which is stabilized by co-valent linkage and hydrogen bonds (159).

Elastin comprises 2 - 5% of the dry weight of the skin, 3Ø - 57% of the aorta and 78 - 8Ø% of the ligamentum nachea. Elastic fibers serves as a barrier in vessel walls, protecting various mesenchymal cells from the serum constituents. It contains a little or no carbohydrate. One third of the amino acid residues in elastin are glycine and one ninth are proline. Elastin contains a very little hydroxy proline and no hydroxy lysine. It is rich in valine. Most of the naturally occurring elastin is insoluble and can't be extracted. Elastins are synthesized mostly in fibroblast (159).

2.1.2.2 TRANSPORT THROUGH BIOMEMBRANES

At one time it was thought that the movement of substances through the membranes was determined solely by concentration gradients, but in contrary to this fact movement against concentration gradient have been observed in most biological tissues or biomembranes. For example, potassium is usually accumulated in the plant and the animal cells to a concentration many times higher than that of the medium surrounding the cell. Such transport requires energy by the cell and has been called **active transport** or metabolically linked transport, while that which responds to concentration gradient is **passive transport**. The energy requires for active transport comes from ATP or other high energy sources. The passive transport is misleading in the case of cells or tissues, because, while the cell may be passive with respect to the movement, no directional movement across cell membrane occurs without the expenditure of the energy by the molecules involved. Kinetic energy accounts for the random movements of molecules and the chemical potential energy of high concentration of a particular substance outside the cell than inside the cell. This gives effective direction to the movement which is dissipated as the molecule of that

substance outside of the cell move into the cell and reduces the concentration gradient of the substances between the outside and inside to the cell. **The term active transport is used in different ways by various authors but the satisfactory definition have not been given as yet** (160).

Some molecules move directly through the pores in the cell membranes, e.g. water and ions of small diameter. There are evidence that other molecules are attached to the carrier with which they form **'complexes'** in the cell membrane. The complex moves the substance across the membrane, liberating on the other side. Since the carrier which shuttles the molecule from one side of the membrane to other, facilitates diffusion, such movement is called **facilitated diffusion. It differs by simple diffusion by kinetics of entry relative to concentration.** The flux through the membrane by simple diffusion increases linearly with increase in the concentrations of the substance, while the flux of the facilitated diffusion levels off with increasing concentration. This means that the carrier sites in the membrane becomes more and more completely occupied untill no additional site can be occupied. There is no further increase in the flux, this phenomenon is known as **saturation. Although the carrier complex requires the**

energy of activation to cross the membrane barrier, less energy is required for facilitated diffusion than for the activated diffusion because of the affinity of the carrier for component (especially lipid) in the membrane. The flux for a given substance is, therefore, greater when a carrier substance is involved than when it is absent. The kinetics of facilitated diffusion resemble with that of active transport but actual mechanism of active transport is still uncertain. The process seems to be quite general in plant cells, animal tissues and micro-organisms. Many suggestions for the mechanism of active transport have appeared in the literature including working model of ion transport. Summaries of these hypotheses including figures and references to other literatures are available (161-163).

At present, we have far more advanced and complicated knowledge concerning the nature of the active transport than what we had some years back. With more advanced knowledge, many issues have been settled but several controversies are yet to be solved. There seems to be general consensus that carrier is involved in both facilitated and active transport and carrier is protein. It seems that carrier proteins is itself not an enzyme but linked to an enzyme or enzymes and

thus energy from ATP or some similar high energy phosphate bond is made available for active transport. Considerable evidence has been accumulated pointing to the Na-pump as prime over suggesting that the movement of other molecule was co-transport but the situation is not general and the problem remains open.

The transport of inorganic ions, organic ions, sugars and aminoacids across biomembranes are gaining importance. An extensive account of the subject of (Na^+-K^+) ATPase and alkali metal ion transport was reported and reviewed by Bonting (164) Heinz (165), Albers (166), Post (167) and Whittam and Wheeler (168). Bonting (164) also reported (Na^+-K^+) ATPase in E.coli cells, sensitive to ouabain. Later on, a new line of approach opened by Skou's Work (169) on Mg^{+2} - dependent membrane ATPase transport on crab nerve. Active Ca^{+2} transport in sarcoplasmic reticulum was reported by Hasselbach and Makinose (17Ø). Bennet and Melamy (171) gathered evidences for two different transport systems for phosphate in E.coli. Gloriux and Scriver (172) also identified two types of transport systems in human kidney, for phosphate transport. Sugar can't pass through membranes by free diffusion. Carrier mediated transport was reported for sugar transport in living membranes. The

transport of glucose into red blood corpuscles occurs by process of facilitated diffusion (Stein (173)), while in other tissues the active transport of sugar requires co-transport of Na^+ (Schultz and Curran (174)). Most of the sugar transport in microorganisms, except that of glucose, is inducible. E.coli. and S. typhimurium apparently transport sugars by three different processes, i.e. facilitated diffusion, active transport and group translocation (Ussing (7), Egan & Morse (175,176) and Simoni (177)).

Transport across biological membranes is also currently undergoing transition from a purely kinetic description familiar to biochemists, to rather esoteric, and yet powerful irreversible thermodynamics description (178). Biological transport processes in terms of irreversible thermodynamics was elaborated much earlier by Katchalsky and collaborators (179,180). Grell and Oberbaeumer (181) published a review in which discussions were made on the membrane protein carrier mediated transport of cations through biological membranes. Fletcher (182) examined the electrical potential differences across the membranes in which active transport of ions occurs, based on the non-equilibrium thermodynamics.

Recently, the transfer of solutes across plasma membrane has been characterized primarily in terms of **kinetics** and **thermodynamic** parameters. The kinetic characteristics suggested that the membrane contains entities called ' carriers' with which hydrophilic solutes, such as, ions, sugars and aminoacids, must form a dissociable complex so that they should more rapidly cross lipid bilayer matrix of the membrane. From thermodynamic consideration it has become clear that the energy for certain transport systems which involve uphill movements was derived from metabolic reactions and that enzymes were directly or indirectly coupled to the 'Carriers'. Chemical information concerning to the nature of the 'Carriers' was restricted to the general conclusions derived from substrate specificities and from the chemical properties of inhibiting agents. In the recent years, the emphasis has been shifting from hypothetical carriers to the identification of actual membrane components involved in the transport. Obviously, membrane proteins are considered vital because most of the transport systems were sensitive to protein perturbants and because certain transport systems involve membrane associated enzymic activities like ATPases, phosphotransferases and others.

The shift in interest from kinetic and thermodynamic to the biochemical approach became possible because of the development of new technologies not only for extracting, separating and purifying membrane components, but also for identifying particular functional components at the time when the membranes dissolved. After isolation, some of the transport proteins may display activities or properties that are related to their function i.e. ATPase activity of cation transport systems, but others have no such endogenous markers. In current days, chemical probes have been proven useful not only in identifying transport proteins, but also in identifying their general arrangements in the membrane. The specific transport systems responsible for the cation transport across red cell membrane, which provides a useful model for examining the use of probes in determining the role of membrane proteins in transport phenomena, were reviewed by various workers (183).

2.1.2.3 BIOPHYSICAL STUDIES ON BIOMEMBRANES

When a membrane possessing fixed charges is in equilibrium with an electrolyte solution, an electrical potential difference is generally established between the membrane and the solution. There has been two entirely different approaches to describe the potential differences.

One approach considers the potential difference to be a **Donnan equilibrium potential**. This approach has been used particularly for studies of ion transport process through the membrane. In other approach, it is regarded as the surface potential i.e. **Gouy-Chapman diffused double layer potential**, which is familiar in colloid sciences. Several modifications on the surface potential approach have been attempted by allowing the surface region to be permeable to electrolyte ions in relation to cell interactions. In spite of these improvements on each of the two different approaches, there remained ambiguity as to the interrelation or transition between the Donnan potential and the surface potential. Ohshima and Ohki (13Ø) have recently shown that **these Donnan and surface potential concepts do smoothly make transition from each other in their potential distributions across a model charged membrane.**

Since most of the biological membranes are surrounded by electrolytes containing various monovalent and divalent ions and these ions may bind to the membrane fixed charge sites, the potential distribution across a membrane having fixed charged layers can be studied in terms of ionic concentrations of bivalent and uni-univalent electrolytes, and ion binding fixed charge sites.

Mond (184) postulated that anion versus cation selectivity might be explained by supposing that the anions diffuse through the regions of membrane containing positive fixed charges which attract anions, but which exclude cations because of electrostatic repulsion. Using a refined version of this model, Passow (185) was able to quantitatively account for the effect of H^+, $SO4^{-2}$, and Cl^- concentrations on the rate of exchange of $^{35}SO4$. This model provided an attractively simple molecular mechanism which was able to account for the most of the measurements of anion exchange carried out prior to the last decade. The advantages and limitations of the fixed charge model in its various forms are thoroughly discussed in several reviews (185-187). Several other models for biomembranes and transport theories have been presented and reviewed by investigators (188-193) to know the physico-chemical behavioural properties of biomembranes and ions transport through them.

Takeguchi and Nakagaki (194) studied the partially selectively permeable, positively and negatively fixed charge membrane. The concentration dependence of two kinds of membranes potentials (i.e. bi-ionic and membrane potentials that arise when charge membranes used to separate two solutions, each of which contains a different

co-ion and same counter ion), have been measured. It has been said that most of the biological membranes bear negative charges, and Na^+ and K^+ ion concentrations are high in the outer and inner phases of the membranes, respectively, i.e. counter ions are different. On this basis, it has also been investigated that bovine lens capsule has positively fixed charges (195). In their subsequent paper (196) emphasis has been given on theoretical and experimental studies on membrane potential arising between two solutions of an electrolytes, separated by Bovine Lens Capsule, *in vitro*. Fan et al. (197) investigated the membrane potential of a basement membrane, by the use of Gibbs-Donnan systems. TMS theory was applied for treatment of experimental data, and charge density and mobility ratios of the ions were evaluated. Umpteen literature were available where membrane potential measurements were made, on the olfactory mucosal epithelial cells, test buds etc. (23). Purice (198) recently used the membranes with large contents of collagens, as a model for electric charge membranes. The transmembrane potential was measured across such membranes for spontaneous diffusion of one or two cationic species and TMS theory was used to compute the junction potential and effective fixed electric charge density. Anion permeability of mammalian red blood

cells with possible relation to membrane phospholipid pattern has been investigated by Deuticke and Gruber (199). The dependence of cell membrane potential upon ion concentrations has been described by Jaffe (200) on the basis of Goldman constant field equation or Hodgkin - Horowicz equation.

Electrokinetic phenomena are currently of considerable interest from the view point of biological transport. Streaming potential measurements across biological membranes have been quite useful in understanding the mode of transport and physiological behaviour of biomembranes. Since electrical potential exists across most of the living membranes, electrokinetic studies have acquired additional significance. Furthermore, the applicability of the methods based on irreversible thermodynamics processes to flows through biological membranes, have also gained considerable attention in recent years.

Since, one of the main characteristics of living cells is their complex compartmentalization. the cell itself and its various internal compartments, are open systems which exchange heat work and matter with the surroundings. The cells are separated from the outside and

each compartment is separated from the neighbouring ones, by lipoproteins membranes. These membranes bear specific mechanism of transport for a great variety of substances including mineral ions. Two different approaches had been taken by Thellier et al. (201) on the specificity mechanism of transport. The first approach was molecular one, where one can separate the various components of the membrane and study their characteristics (molecular weight, shape, affinities etc.), and possibly try to insert them into artificial or real membranes. The second important approach was the macroscopic or global one, where one studies the transmembrane exchanges using entire systems. The measurements then are electrical potential differences, concentration gradients, etc.The effect of a variety of disturbing agent can also be studied such as temperature, pH or ionic strength, modification, chemical inhibitors etc. Most of the work available are on skin membrane. Ussing (8) summarized his work on water and electrolyte transport across frog's skin, considering the epithelial layer of frog skin as a two membrane systems, there can be three possibilities of sodium transport across it, the coupled-Na transport exchange, true electrogenic sodium transport and coupled Na-anion chloride pump. In the previous paper, Ussing (9) specified that ion transport mechanism may point to specific ways to introduce radioactive isotopes.

Simons (2Ø2), later on, applied the irreversible thermodynamic methods to the problem of particle flow through biological membranes. The formula has been derived for the uni-directional fluxes and flux ratio of permeant species under the conditions where there is a coupling between flows and metabolism. The usefulness of this flux ratio equation has been shown under appropriate conditions for providing evidence antagonistic to the hypothesis of active transport of permeant species. The conditions under which the use of tracers should lead to accurate estimation of unidirectional fluxes have been specified. Since then, various studies have been performed based on **fluxes/forces**. Ussing (1Ø) gave a flux ratio equation that discloses the contribution of active transport or interaction in the movement of electrolytes or non electrolytes in biological membranes by determining the electrochemical potential difference. Watlington and Jesse (2Ø3) measured the Cl^- flux across frog skin. Ronald et al. (2Ø4) studied the chloride transport across frog skin. Larsen and Rasmussen (2Ø5) carried out investigation in order to know the role of membrane potential for chloride transport in frog skin. The electrophysiological characterization of ion transport (Na^+ and Cl^-) across isolated frog skin was also studied (Rafael and Daniel

(2Ø6)). Later on, Ussing and Karen (11) and Karen & Ussing (2Ø7) determined the electromotive force of active sodium transport in frog skin epithelium with the help of steady state flux ratio equation. Walser (2Ø8) and Walser et al. (2Ø9) measured the flux of sodium and chloride ions across toad blodder in the absence of ouabain. Chen and Walser (21Ø) also measured the flux of sodium and sulphate ions in toad bladder treated with sufficient ouabain to inhibit active sodium transport, at the potential between Ø to 1ØØ milli volts. Chock and Titus (1Ø7) described the mechanism of ion transport through biological membranes and discussed the effect of alkali cation on enzymic activity and sodium dependent transport of polar organic substances. Meyer et al. (211) carried out positional analysis of electrolytes membrane potential using **in vitro** preparation of rabbit distal ileum. Person and Spring (212) studied the permeability properties of epithelial connective tissue of Necturas gallbladder by measuring electrical resistance and dilution potential. Ripoll et al. (213) described the ion transport through biomembranes with biological implications of the results, while Jose et al. (214) studied the ammonia transport in turtle's urinary bladder. Naidu (215) recorded the transpepithelial potential difference and diffusional water up take in ventral pelvic patch of the skin of **Rana**

cyanophlyctis and **Bufo melanostictus**. A study was also under taken to examine the role played by various transport systems in over all transport of chloride ions across frog skin (216). The Ussing's method was adopted to study the basal electrolyte transfer as well as the event occurs upon odorant stimulation in frog olfactory mucosa (217).

Shukla (218) and Shukla et al. (219) have carried out the hydrodynamic permeability, electro-osmotic permeability; and streaming potential measurement for urea and urine solution across urinary bladder membranes, based on the method of non-equilibrium thermodynamics. In their latter studies (22Ø) hydrodynamic permeability of aquous solution of glucose, fructose, galactose, lactose, and sucrose and their mixtures and aquous solution of urea, thio-urea, acetamide, glycine and methanol, across urinary bladder membranes of goat, were measured, in terms of non-equilibrium thermodynamics. It resulted from the experiments that the urinary bladder membrane behaves as an anisotropic way. Irreversible thermodynamic approaches to membrane transport was also reviewed by Sitaramam (178). Hydrodynamic permeability measurements on water and on aquous solution of D. glucose and sucrose of various concentration through frog skin membrane, were also made.

The conductance of the membrane equilibrated with permeants was also measured (221). The dependence of the permeability coefficient and membrane conductance on the concentration was discussed and the results inferred that the skin membrane behaved anisotropically so far as the hydrodynamic permeability was concerned.

Rosoff et al. (222) measured the unidirectional Ca^{+2} fluxes **in vitro** across urinary bladder and colon of B. marinus in the absence of electrochemical gradients. A net Ca^{+2} flux was observed in each tissue but with different polarity. Fluxes was observed from mucosal to serosal side of colon and vice-versa in the urinary bladder. A study was also conducted to determine the type and activities of carrier proteins which transport chloride ions in pig jejunal brush border membranes, with emphasis on the properties of chloride ion conductance activity in vesicles prepared from these membranes (223). The dilution and bi-ionic potentials across isolated rabbit corneal epithelium have also been measured to learn about the ionic selectivity of its interacellular junctions (224). Transepithelial electrical potential differences induced by glucose were measured in rat small intestinal under experimental diabetic conditions (225). Human eccnine sweat glands were isolated and the potential differences across the

basolateral membranes were determined using levelled microelectrode (226).

2.1.2.4 BIOCHEMICAL STUDIES ON BIOMEMBRANES

Proteins and Lipids

The family of proteins grouped under generic name of collagen is the main component of all biological tissues. Also other constituents of extra cellular matrix, as elastin, proteoglycans and glycoproteins, are crucial in the maintenance of mesenchyman viscoelastic properties. Chemical studies revealed that extracellulor macromolecules of connective tissue may interact with lipids and be responsible for the lipid depositions of connective tissue (227,228). There are various reports on direct effects of lipids on collagen producing cells and tissues (229-231). The organization of collagen fibers, and the chemical, biophysical and biological properties of tissues appear to be dependent on tissular lipids.

Lipids and proteins are quantitatively major components of biological membranes. It has been generalized that the membranes that behave mainly as a barrier, contain highest lipid to protein ratios (232). The amount of cholesterol mostly present in the animal membranes, relates to their barrier function. The nature of phospholipids were

also a property peculiar to each membrane types. The role of proteins in transport system was also well understood. Therefore, to characterize a membrane, it is obvious to isolate and identify the different component of the membranes.

Membranes of erythrocytes of different ages have been separated and the lipid pattern was studied in normal and diabetic state (233). Cholesterol, lipid phosphorous, free fatty acid, triglycerides, phosphatidylethanolamine and sphingomyelin showed a gradual increase in their concentration during aging of the erythrocytes. Bansal et al. (234) studied the changes in the content of phospholipids, cholesterol and sialic acid, and the activity of enzyme acetylcholine esterase in the normal and alloxan diabetic monkeys. It has been found that all the above constituents decreased significantly in diabetic state in comparison to normal erythrocyte membrane. Phospholipids have been identified by thin layer chromatography (TLC) in pericardial fluid in experimental animals e.g. dogs; and found that sphingomyelin, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidylserine were present, in which phosphatidylcholines predominates (Hill and Butler (235)).

A study on the lipid content and composition of bovine retinal outer segment's fragments have been done (236). Almost half of the retinal outer segment fragments have lipid by weight basis, most of the lipids are phospholipids, having, phosphatidylcholine, - ethamolamine, and -serine in majority. But significant amount of -inositol and sphingomyelin are also present. The fatty acid portion contained an usually high concentration (approx 50%) of highly unsaturated chain fatty acid. The chemical composition of sarcolemmas isolated from rabbit skeletal muscle has been also studied in detail (237). Ketterer et al. (238) analysed the lipid and protein component of rat bladder. the principal lipid component was found as cholesterol, phosphatidylcholine, phophatidylethanolamine and cerebroside while the amino acid composition differed from other cell substraction in having an usually high proline content. Rosenbloom and Elsbach (239-241) have studied on urinary bladder of toad and found that this membrane was composed of mainly collagen fibers, thin smooth muscle layer at mucosal side, a serosal layer and few blood vessels. Latter investigation (242) has revealed that the bladder membrane functions as **molecular sieves**.

A series of review work has also been published, in which discussions were made on determinants of biomembranes,

its motional dynamics, structural interactions of lipids and proteins and preservation of membranous structure by carbohydrate and phospholipids polymers (243). Review work were also available on the possible molecular configuration of membrane lipid and protein, their interrelation in different molecular interactions (244). Recently, Maroux et al. (245) published a review on the molecular structure of brush border membranes of intestine, the cytoskeleton organization was also described and membrane components were discussed with emphasis on its glycoproteins.

ATP-ases

Over the last decades, numerous investigations have provided a substantial body of evidence for the hypothesis that membrane bound adinosine triphosphatases (ATP-ases) are the biochemical expression of certain transport mechanisms across various membranes. In studying the relation between ion transport and ATPases, there is a need to determine the several membrane bound ATPases which are effective in one way or other in different membrane systems.

Abundant literature is available regarding the isolation and characterization of different ATPases. Skou's work (246) studied the effect of enzymic activity exerted by cations present in the tissues. Bonting and Caravaggio (247)

and Bonting and Canady (248) demonstrated the presence of (Na^+ - K^+) ATPase activity in toad bladder. Mizuno et al. (249) estimated the lipid composition and (Na^+-K^+) ATPase activity in rat lens. Peter et al. (25Ø) estimated the different ATPases, proteins and sugars in kidney outer medulla. Hasting and Skou (251) determined the (Na^+ - K^+) ATP ase activity by measuring the inorganic phosphate released by enzyme during incubation time in presence and in absence of ouabain. The membrane ATPase activities in the rat pancreas has been studied to investigate the possible role of these enzymes in HCO^-_3 secretion in pancreas (252). The enzymic activities, lipid content and electronmicroscopic features of isolated plasma membrane from cardiac muscle cells have been investigated by Barr et al. (253). Pierce and Dhalla (254) examined the activities of various enzymes including (Na^+ - K^+) ATP ase, associated with the sarcolemmal membrane of heart, in control and diabetic condition. Alteration in membrane lipid content are known to effect membrane permeability and enzymic activities. Studies have been carried out where the involvement and participation of membrane lipids in the defect of (Na^+ - K^+) ATPase activity has been investigated (255). The effect of certain substances on the enzymic activities has been also described, e.g. glucagon and

epinephrine inhibits the enzymic activity while insulin counteract this effect (256). Altered membrane bound ATPase activities in uncontrolled diabetes after insulin administration has been investigated in experimental diabetic rats at different parts of the body, e.g. nerves, heart, glomeruli, medula and cortex (257).

Procedure has also been described for the analysis of membrane bound (Na^+ - K^+) ATPase with the use of electronmicroscope after thin, sectioning, staining and freez fractures (258). Cantley (259) presented a review about structure and mechanism of (Na^+ - K^+) ATPase from a variety of sources and mechanism of transport mediated by this enzyme. Joergensen (26Ø) reviewed and discussed the purification of membrane bound (Na^+ - K^+) ATPase within mammalian kidney outer medulla. Milutinovic and Molnar (261) performed the preliminary determination of (Na^+ - K^+) ATPase in erythrocyte membranes of rats as well as experimental determination of these ATPase in human erythrocytes ghost.

In the year 1961, Dunham and Glynn (262) observed another ATP ase activity in human red cell membranes which is stimulated by low concentration of Ca^{+2} and Mg^{+2}. Evidences for vectorially active transport of Ca^{+2} from inside of the erythrocyte to the surrounding medium were

given by Schatzmann and Vincenzi (263) and Weiner and Lee (264). Since then many efforts have been made to isolate this enzyme (265,266). The presence of Ca^{+2} - ATPase have been demonstrated in plasma membrane of embryonic chick fibroblast, in the cells of rat kidney cortex, guinea pig placenta, egg cell of fresh water fish and sea urchins. The existence of Ca^{+2} - stimulated ATPase in rat liver plasma membrane and (Ca^{+2} - Mg^{+2}) - ATPase in mouse liver plasma have been also investigated. The existence of outer complex enzyme system consisting of (Ca^{+2} - Mg^{+2}) ATPase, (Ca^{+2} - Mg^{+2} - Na^{+}) ATPase and (Ca^{+2} - Mg^{+2} - K^{+}) activated ATPase has been also demonstrated (266). Schatzmann (267) examined the dependence of activity of (Ca^{+2} - Mg^{+2}) - stimulated membrane ATPase on the ATP concentration at the different and constant Mg^{+2} concentrations with the same constant Ca^{+2} concentration in both instances. Roufogalis (268) reviewed in detail the mechanisms by which the red cell maintains an asymmetric distribution of Ca^{+2} against an electrochemical gradient. Emphasis was also given to the physiological significance of regulation of Ca^{+2} pump by calmodulin, a Ca^{+2} binding proteins in the cytosol of red blood cells. It has appeared that estimates of Ca^{+2} affinity of the Ca^{+2} pump in R.B.Cs, varying from micromolar to millimolar range, depend on the preparation used for

transport studies as well as composition of assay medium. The (Ca^{+2} - Mg^{+2}) ATPase which is the biochemical expression of the Ca^{+2} pump, varies in membranes isolated from different procedures (269). The calcium dependent magnesium stimulated (Ca^{+2} - Mg^{+2}) ATPase was associated with Ca^{+2} transport in sarcoplasmic reticulum and Ca^{+2} - ATPase activity was dependent on membrane lipids (27Ø). The EGTA effect on Mg^{+2} dependent, Ca^{+2} stimulated (Mg^{+2} - Ca^{+2})-ATPase in human erythrocyte membranes was studied by Al-Jobre and Roufogalis (217). Advances in purification of plasma membrane vesicles together with the measurements of Ca^{+2} transport in isolated cells or tissues were also being done to provide information about the nature of plasma membrane Ca^{+2} transporters including (Ca^{+2} - Mg^{+2}) ATPase in a wide variety of cell types (272).

Trace elements

The importance of trace metal ions to the vital functioning of living organisms and, hence, their health and well being, have become increasingly apparent. The metabolism and transport of metal ions and their complexes are being studied, and new methods of complicated natural structures and process are being devised and tested. The focal point of attention is the connection between chemistry

of metal ions and their role for life. It is hoped that this would break down the barriers between the historical spheres of chemistry, biochemistry, biology, medicine and biophysics, with a good deal of future outstanding discoveries to be made in the interdisciplinary areas of science (273).

It is now becoming increasingly evident that trace metals as an integral parts of tissues and biological fluids, are one of the many homeostasis mechanisms regulating the reactivity of the tissues and cells. Several of these actions are mediated through membrane bound enzymes. However, the investigations in this direction are confined to the study of a few enzymes (e.g. ATP ase etc.) mainly in the liver tissues, blood cell and sarcoplasmic reticulums etc. Furthermore, the effect of interactions of these trace metals on the enzymes have not been fully exposed. Recent studies have revealed that changes in trace metal concentration are closely related to a number of disease processes in human systems. It is also well established that metals are important constituents of biological membranes. They may affect the biophysical properties of biological membranes by virtue of their electrophysiological effects. They are also important

component of metallo-enzymes. Therefore, they influence the active transport through membranes by modulating the various membrane enzymes. As membranes controls the active movements of fluids, it is expected that alteration in metal environments will not only affect the electrophysiology of biological membranes but will also influence the active transport through them by altering the function of various membrane bound enzymes of which the metals are important constituents. It is, therefore, very relevant to examine the metal composition of the biomembranes, as well as the effect of altering the milieu of trace metals elements on the electrophysiological properties of biological membranes.

Iron was first element to be determined as essential in the 17th century. Other elements, such as, Cu, Mn, Zn and Co were determined to be necessary for tissues in late 192Ø's and 193Ø's (274). More recently, the necessity of Si, Sn, V, Ni, I, Mo, Hi, Cr, Se, B and As have also been established (1). the macroelements, C, N, Na, O, Mg, P, S, Cl, K, Ca and H can be easily analyzed by standard methods. The trace elements can be classified as toxic or essential, though in practice one element can be both, depending upon concentrations. Morrison (275) has recently reviewed the status of elemental trace analysis of biological materials in detail.

The trace elements studies have been carried out on in several disease states. the effect of different concentration of trace elements injected intraperitoneally in the experimental animals have also been investigated (276,277). The trace metal ions have a non-homogenous distribution in CNS, and this can be interpreted as an expression of functional differences at a given moment. Alteration in their concentration can lead to change in neuronal function. Zn and Cu are well established trace metals, essential for the stability of macromolecules and enzymic function of key metabolic significance. Rehaman et al. (278) determined the changes in concentration of Zn, Cu and Pb in blood, spinal cord and different parts of brain after Zn administration in rabbits. Irving Lyon (279) examined the effect of Cu^{+2} on isolated frog skin.

Zinc is essential for the normal growth, and it influences the function of the various organs in the body. Liver, pancreas and kidney play a leading role in the regulation of homeostasis of Zn in the body. An increase in the tissue concentration of Zn was reflected by enhanced levels of low molecular weights isoproteins of metallo-thioneins (28Ø). Interest in lithium chemistry in biological system was generated because of its beneficial effect in control of manic-depressive psychosis.

Speculation as to the origin of this action had been wide spread with one major suggestion being that it arised from a specific Li^+-phospholipids interaction. Significantly important interaction between Li^+ and phospholipids membranes, particularly those with negatively charged surface, has been reported (281). The effect of barium on the basolateral membrane potential of the isolated perfused rabbit proximal tubule has been studied by Beck et al. (282). Berke et al. (283) analyzed the plasma and membrane bound Zn and lipid composition in mice. The Zn and lipid metabolism have appeared to be altered in diabetic state but membrane bound Zn did not seem to be affected. The influence of dietary Cu on lipid composition and fluidity of liver plasma membrane has also been determined (284).

2.1.2.5 PERITONEUM

Peritoneum is a readily available biological membrane. It is the largest serous membrane found in the body. Now-a-days peritoneal dialysis is being extensively used to treat the patients suffering from renal and non-renal diseases. The stomach and intestine in the body are suspended in the abdominal cavity by mesenteries, and their surface is covered by a moist serous membrane known as the serosa or peritoneum, which permits these organs to slide freely over

one another within the cavity of digestive tract (14). There are two, parietal and visceral, portion in the peritoneum. The free surface of the membrane is smooth, covered with a layer of flattened endothelium and lubricated by a small quantity of serous fluid. The attached surface of peritoneum is rough being connected to viscera. The visceral and parietal layers of peritoneum are in actual contact and the potential space between them is known as peritoneal cavity consisting of greater sac and lesser sac. The peritoneum differs from other serous membranes of the body in presenting a much more complex arrangements (285).

The histological features of peritoneum membrane resemble with that of duramater and pericardium. All these are serous membranes. Detailed histology of serous membrane are available in literatures (286-288). The serous membrane are thin layers of loose connective tissue covered by a layer of mesothelium. When the membranes are folded, forming the omentum or the mesentery, both free surfaces are covered with mesothelium. The cavities lined by serous membrane always contain a small amount of liquid, the serous exudate. The cell in this exudate originates from the serous membrane. All the elements of loose connective tissue are found in serous membranes, such as, the mesentery. Because, they are very thin and require no sectioning. The

mesenteries have been the favourite site for the microscopic study of loose connective tissue. A mesentery contains a loose net work of collagen and elastic fibers, scattered fibroblasts, macrophages, mast cells and varying numbers of fat cells.

Physiologically, the most important and histologically the most interesting part of the serous membrane in mammals are omentum. The membrane is pierced by innumerable holes and thus reduced to a fine lace like net formed by collagenous fiber bundles covered by mesothelial cells. Such thin fenestrated areas have a few or no vessel. In the thicker areas where the omentum is a continuous sheet, macrophages are numerous. There are also many small lymphocytes, plasma cell eosinophilic, leukocytes and mast cells. The number of lymphocytes and mast cells vary considerably in different animal species (287).

The omentum in man extends downward from the greater curvature of the stomach like a loose curtain or veil over the intestine and is of great clinical significance in the limitation of disease processes in the abdominal cavity. In addition to the protection afforded by the adhesion of the omentum, the free cells of its connective tissue constitute an important mobile reserve to combat infections in the peritoneal cavity (287).

The connective tissue functions in mechanical support, exchange of metabolites between blood and tissue, storage of energy in adipose cells, protection against infection, and repair after injury. Fibrous components are most important for its mechanical role and their abundance distribution adapted to the local structural requirements. Delicate network of reticular fibers support the basement lamina of epithelia. Connective tissue plays a significant role in the nutrition of the other tissues that it surrounds and permeates. It is evident that all substances reaching the cell and tissue from the blood and all the products of metabolism are returned to the blood and lymph must pass through a layer of connective tissue.The metabolites are believed to be diffused through the aquous phase of gelatinous ground substance or along the thin films of fluid coating the fibers. The exchange of material is probably influenced by the viscous properties of the ground substance. The poly electrolyte properties of its glycosaminoglycans suggest that the connective tissue ground substance may also participate in maintaining water and electrolyte balance (287).

Transport Studies on Peritoneum

Abundance of literature is available on peritoneal membrane transport which speaks volumes of its importance.

It will be too extensive to cover the extensive literature available describing the various studies on peritoneum. Knapowski et al. (289) studied the effect of ethacrylic acid, water and sodium transfer across human peritoneal membrane. Later on, Feder and Knapowski (290) studied the net Na^+ transfer in parietal peritoneum covering the diaphragm and anterior abdominal wall and observed the effect of furosemide on such transfers. They found that the mesothelial cells of parietal peritoneum reacts to pharmacological agents with altered transport properties. Simon et al. (291) investigated the flux in in vitro rat peritoneum preparation mounted in Ussing chamber, unidirectional flux from vascular to mesothelial side for sodium, potassium and calcium has been found to be greater in diaphragmatic portion of the peritoneum than anterior abdominal wall portion. The usefulness of this model in the investigation of function of peritoneum in dialysis has been discussed. Furosemide effect on uric acid transperitoneal transport was investigated and it has been concluded that furosemide had diverse effect on transperitoneal uric acid transport depending not only in the type of rabbit peritoneum (mesentery, ileal, parietal etc.) used, but also on the side of the membrane it was applied (292). Nolph (293) has presented a discussion on the causes and control

of sodium and potassium losses during peritoneal dialysis. To investigate the osmotic barrier characteristics of peritoneal membrane during condition similar to the peritoneal dialysis in man, net transperitoneal fluid movement was measured in cats following intra-abdominal placements of isotonic saline and hypertonic solution of sodium chloride, glucose, raffinose and insuline (294). It was concluded from the results that peritoneum is a highly selective membrane with restrictive properties comparable to those reported for continuous capillary beds. The effect of alloxan induced diabetes-mellitus on the permeability performance of peritoneum was also investigated by Zimmerman et al. (295). Purification of plasma membrane were made from the guinea pig peritoneum macrophages by centrifugation. The subcellular fractions obtained were subjected to biochemical and ultrastructural analysis (296). To know whether viscera contributes to the systems of membrane used in dialysis, the dialysis rates studies were performed in rats comparing control with eviscerated ones (297). Rats without intestinal viscera had mass transfer coefficient equal to or greater than those of the rats with viscera. In order to ratify the quantitative description of the peritoneal transport of drug, a kinetic model was proposed by Nakashima et al. (298) based on hydrodynamic

pore theory of transcapillary exchange. Quinolone transport across peritoneal membrane was also studied and found to be diffusion limited (299). Wallaeys et al. (3ØØ) discussed the possible mechanism involved in the transfer of chromium from dialyzate to blood during peritoneal dialysis. Wideroe et al. (3Ø1) has studied the peritoneal membrane water transport in different clinical situations. The results were based on the mathematical modelling used to stimulate structural alteration in peritoneal membrane. Dobbie et al. (3Ø2) under took a study to investigate the possibility that the peritoneum is capable of synthesizing the phosphatidylcholine, a lubricant surfactants, in an amount similar to that produced by pulmonary alveoli. Electron microscopy of transparent mesentery as a test tissue showed that the mesothelium constitute bulk of the cell population, possibly engaged in producing phosphatidylcholine. These findings were of significant relevance to the role of surfactants phospholipids in the physiology of peritoneal dialysis. Recently a biomaterial has been prepared, suitable for the use in medical devices, comprising the peritoneum tissue which has been chemically treated to crosslink the collagen in the tissue, rendering the tissue more stable, less antigenic and sterile (3Ø3).

Ultrastructural Studies on Peritoneum

The ultrastructural studies of peritoneal mesothelial cells have been described in detail by Lentz (3Ø4). Comparison of ultrastructure and histochemical characteristics of surface cells of ovary and peritoneal mesothelium were made (3Ø5). Normal surface cells of ovary tend to be cuboidal or columnar in shape while mesothelial cells were found to be flat. Surface cells of ovary bears cilia while mesothelial cells do not. Cases of peritoneal fibrosis induced by beta adrenoceptor blocking agent practolol have also been studied (3Ø6). Histological and scanning electron microscopical specimens witnessed the presence of a thick layer of connective tissue with collagen and fibroblasts. Evidence of mesothelial cells with characteristics microvilli was found to be absent. Bulberini et al. (3Ø7) observed the surface of mesothelial cells of different peritoneal areas in rat by scanning electron microscope (SEM).

CHAPTER-3

MATERIALS AND METHODS

3.1 BIOPHYSICAL STUDIES

The peritoneal membrane of buffalo(Bof. bubalis) aging between 18-24 months was taken out from the local abattoir and immediately submerged into ice cold Ringer's solution (3Ø8,3Ø9) of pH 7.4 ± Ø.2 for the preservation of the membrane tissues. The following composition in gms liter for Ringer's solution was used :

NaCl, 8.ØØ; KCl, Ø.2Ø; $CaCl_2$, Ø.2Ø; $MgCl_2$, Ø.1Ø; $NaHCO_3$, 1.ØØ; Na_2HPO_4, Ø.Ø5, and glucose, 1.ØØ.

The diagram of the apparatus used in the measurements of membrane potentials is shown in Fig. 1. It consists of two half-cells. The vertical female joints A_1 and A2' attached to the each half cell for pouring of electrolyte solutions and calomel electrode B_1 and B_2.

The peritoneum was washed with deionized water several times to remove any traces of Ringer's solution. The membrane was then cut into disc type and installed between two flanges of Pyrex glass cell of the apparatus. The thickness of the membrane is Ø.16 ± Ø.Ø1 cm and effective cross sectional area fitted into the glass cell are 3.Ø2 cm^2. The electrical potentials difference arising across the membrane were measured with Osaw vernier potentiometer (CAT No. 37ØØ1) by maintaining a ten fold

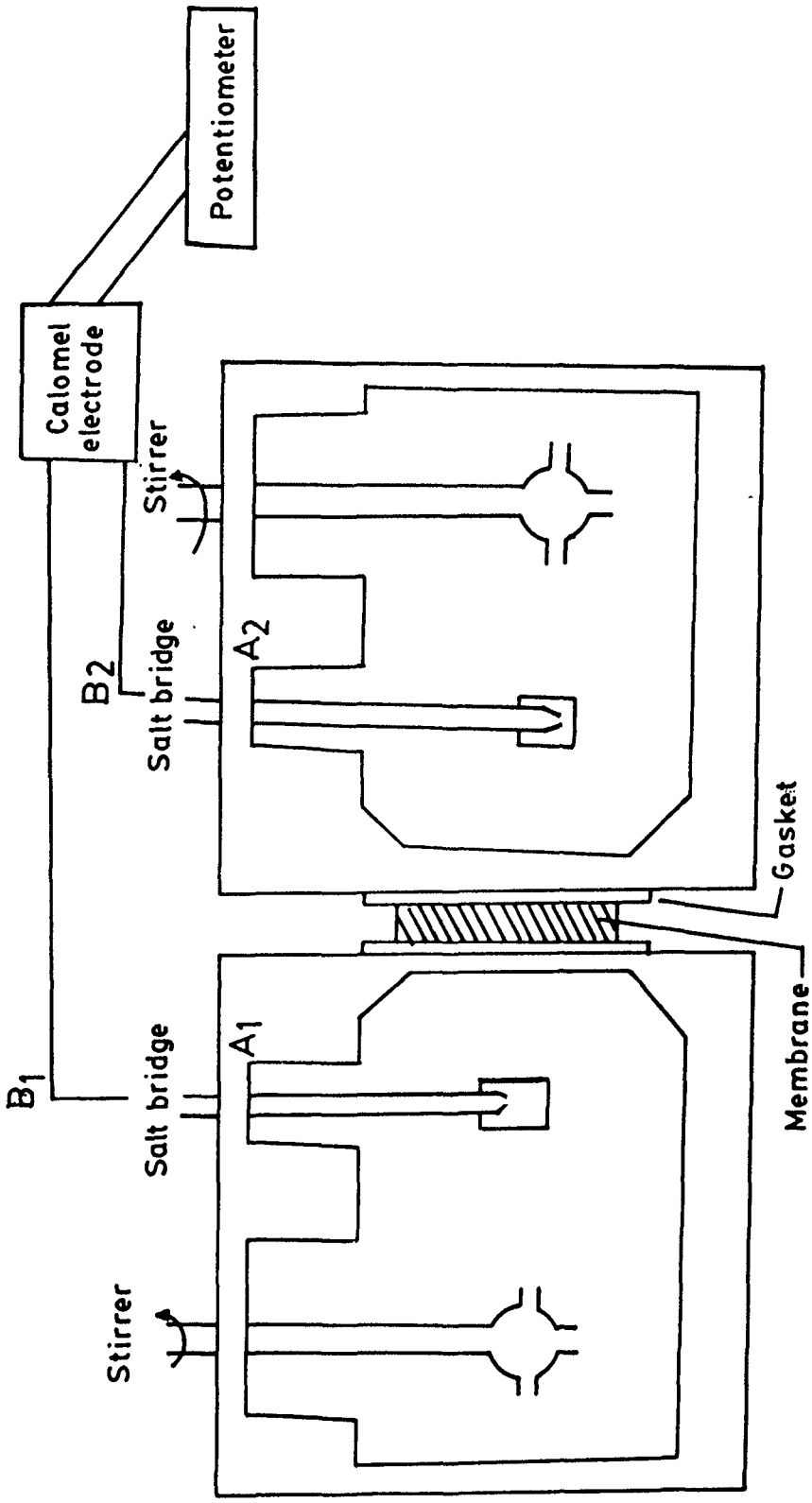


Fig.1 : Schematic diagrame of cell used for the measurement of membrane potential.

difference in electrolyte concentrations, such that C_2/C_1 =1Ø, in the range of 1 x $1Ø^{-4}$ to 1 M. The solutions filled in both the half cells were vigorously stirred by a pair of magnetic stirrers. The various salts solutions (NaCl, KCl, NaF, KF, $KNØ_3$, NH_4Cl, $CaCl_2$, $MgCl_2$, Zn Cl_2, $MnCl_2$, $CoCl_2$, $CuCl_2$ Na_2SO_4 and $CrCl_3$) were prepared from AR Grade reagents (B.D.H., India), in deionized water. The whole cell assembly was immersed into thermostatic water bath maintained at 25 $\pm$ Ø.1° C temperature.

The potential developed by setting up a concentration cell of type described by Michailis (136), Sollner and Gregor (137), Marshall and Ayers (138) and Siddiqi et al. (32-36), was taken as a measure of membrane potential. The electrochemical cell of type (A) was set up for the membrane potential measurement.

S.C.E.	Solution	Membrane	Solution	S.C.E
	C_1		C_2	

$$C_2 = 1Ø\ C_1$$

(A)

where S.C.E. is standard calomel electrode.

The same electrolyte solution with different concentration was used on both sides of the membrane. The

dilute side was taken as negative. The experiments were repeated with fresh solution of electrolyte and maximum potential attained, was recorded, for 3Ø-45 minutes with each set .

3.2 Biochemical Studies

The samples of peritoneum tissue of buffalo were collected from the local abattoir of Aligarh. Collection, mechanical cleaning and handling of the peritoneum were performed with the help of scissor and silica knife. The tissues were freed of any fat debris and the different biochemical studies were carried out with fresh peritoneum tissue samples without any preservation treatment.

Lipid/Protein isolation

The lipids were extracted from the tissue samples by applying the method of Folch et al. (31Ø) using chloroform-methanol mixture in the ratio of 2:1 (v/v). For estimation of different Lipid Components separate methodologies were adopted. Free fatty acid (FFA) estimation was performed with the help of Mosinger's method (311). Cholesterol content was evaluated according to the method developed by Zlatkis et al. (312). Phospholipids were determined as described by Bartlett (313) and modified by Marinetti et al. (314).

Triglycerides were estimated by the procedure of Zilversmit et al. (315).

Protein content was determined by Lowry's method (316) with bovine serum albumin (BSA) as a standard.

Phospholipids composition of the samples of peritoneum tissues were determined by using two dimensional thin layer chromatography (317,318). Thin layer plates were spread with a spreader. Absorbent for five plates contained 2Ø gm silica acid (Silica gel, H., Sisco, India) suspended in a solution of magnesium acetate (1.5 gm per 6Ø ml water). Chloroform-methanol-amonia (65:25:5 by vol.) was used in the first dimension and chloroform - acetone - methanol - acetic acid-water (3:4:1:1:Ø.5 by vol.) in the second. This system provides complete separation of all observable peritoneum tissues phospholipids and was, therefore, used for quantitation. After chromatography the plates were air dried and phospholipids were observed by charring with 3% formaldehyde in concentrated H_2SO_4 solution. The spots which appeared on the plates after charring were transferred quantitatively to digestion flasks and the phophorous contained in each spot was determined by the method of Bartlett (313) and Marinetti et al. (314). Identification of

the phospholipids were made on the basis of known migration of phospholipids in the two dimensional chromatography.

Five to six different samples were considered for each analytical determinations.

ATP-ase assay

A portion of the peritoneum tissue was dissected out from the same experimental animal. It was then rinsed and collected in 9% glucose solution and was cooled in ice. The tissue was then minced with scissors in a pestle and was homogenized in ice cold glucose solution in a Teflon homogenizer with 3-4 strokes at 85Ø revolution per minute. The homogenate was filtered and filterate obtained was centrifuged for 1Ø minutes at 5ØØxg to remove the cell debris. Protein estimation and ATP-ase assay were performed in suspension. Protein concentration was estimated by the method of Lowry et al. (316) with BSA as a standard. The assay of inorganic phosphates was done with the method recently propounded by Raess and Vincenzi (319) where the pi and protein assays provide the basis for simple and sensitive determination of ATP-ase activities.

The ATP-ase incubation media contained the following component in a final volume of 3 ml: 3Ø µl of membrane

suspension, 18Ø mM buffer (Tris HCl, pH 7.1), 8ØØ mM NaCl, 15Ø μM Kcl, 3ØμM $MgCl_2$, 2μM $CaCl_2$, 1 mM Ouabain, 1 m M EGTA (Ethylene glycolbis - (β-amino-ethyl ether) N, N′ - tetraacetic acid) and 3mM adinosine triphosphate (Na_2 ATP), pH 7.1; EGTA is present as a Ca^{+2} sink. Incubation medium component, were added in concentrated solutions. Deletion of one or more components yield the specific medium for various ATP-ases as shown below:

Tubes Nos./ Components	1	2	3	4	5
Buffer	No	Yes	Yes	Yes	Yes
NaCl	No	Yes	Yes	Yes	Yes
KCl	No	Yes	Yes	Yes	Yes
$MgCl_2$	No	Yes	Yes	Yes	Yes
EGTA	No	Yes	Yes	Yes	No
Membrane suspension	Yes	No	Yes	Yes	Yes
$CaCl_2$	No	Yes	No	No	Yes
Ouabain	No	Yes	Yes	No	Yes
ATP	No	Yes	Yes	Yes	Yes
H_2O	Yes	Yes	Yes	Yes	Yes
Activities	Membrane Blank	Reagent Blank	Mg^{+2}-ATPase + Blank	(Na^+-K^+) ATPase+Mg^{+2} -ATPase	Ca^{+2}-ATPase +Mg^{+2}-ATP-ase

Tube 1 Contains no Pi (Inorganic Phosphate)

Tube 3-2 = Mg^{+2} - ATP-ase activity

Tube 4-3 = (Na^{+} - K^{+}) - ATPase activity

Tube 5-3 = (Ca^{+} + Mg^{+2}) - ATPase activity

The three ATP-ases i.e. Mg^{+2} - ATP-ase, (Na^{+}-K^{+}) ATP-ase and (Ca^{+2}-Mg^{+2}) ATP-ase can be determined in a total of five tubes including the appropriate blanks. The tubes were preincubated for 5 minutes at 37°C before adding the ATP. All the chemicals used were supplied by Sigma Chemical Co., USA. Tubes 1 and 2 represent a membrane blank and substrate reagent blanks respectively. In our case, tube 1 has no pi contamination. The sum of Pi in tube 1 and 2 was deducted from tube 3 to obtain Mg^{+2}-ATP-ase. The values obtained with tube 3 was deducted from the values of tubes 4 and 5, respectively, in order to obtain specific (Na^{+}-K^{+}) - ATP-ase and (Ca^{+2}-Mg^{+2})-ATP-ase activity values.

The incubation time was 6Ø minutes at 37°C. The reaction was started with an addition of ATP to appropriate tubes. The incubation period and enzyme activities were

terminated by addition of 1Ø% SDS (w/v) solution. The use of SDS free of Pi eliminates the necessity of centrifugation to remove precipitated protein.

For determination of inorganic phosphate the optical density was measured using 'spectronic-2Ø' spectrophotometer at a wavelength of 82Ø nm. Acid molybdate solution (13Ø ml concentrated H_2SO_4, 25 gm Ammonium molybdate in two liters); 3.33% SDS (w/v) and 9% ascorbic acid (w/v) was used as reagent. A phosphate standard curve was used for calibration.

Trace Elements determination

(a) Processing of membrane tissues

Tubes (corning glass) of 25 ml capacity were soaked in chromic acid overnight. Thereafter, it was washed with double distilled water to be soaked in 1Ø% HNO_3 (v/v) for another twentyfour hours. Then the tubes were cleaned with double distilled water and dried in oven to make them metal free.

A portion of peritoneum membrane tissue dissected out from same buffalo was dried, weighed and kept in a mixture of concentrated HNO_3 and H_2SO_4 in the ratio of 4:1, separately in precleaned tubes.

(b) Digestion of tissues

The tissues were digested in a Kjeldhal apparatus at 60°C for a couple of hours. Hydrochloric acid (1% strength) was added and the volume was made upto 10 ml. This solution was then subjected to atomic absorption spectrophotometry.

(c) Atomic Absorption spectrophotometry:

Perkin Elmer model 303 atomic absorption spectrophotometer equipped with boiling burner and Null recorder read out accessory was used. The operating condition for different trace elements studied herewith were determined. The operating parameters for maximum sensitivity for different trace elements are given as below:

Elements	Wave length (nm)	Slit width (nm)	Lamp current (mA)	Automiz -ation	EHTgain (V)	Scale Expansion	Integ ration time (second)
Ca	422.7	0.5	3.0	Air-Acet.	-484	1	0.3
Zn	213.9	0.5	5.0	-do-	-420	1	0.3
Fe	248.3	0.2	7.0	-do-	-480	1	0.3
Mn	279.5	0.2	5.0	-do-	-496	1	0.3
Pb	283.3	0.2	5.0	-do-	-480	1	0.3
Cu	324.7	0.5	3.0	-do-	-364	1	0.3
Cd	228.8	0.2	3.0	-do-	-368	1	0.3

Cr	379.5	Ø.2	6.Ø	-do-	-368	1	Ø.3
Mg	285.2	Ø.5	3.5	-do-	-344	1	Ø.3
Ni	232.Ø	Ø.2	3.5	-do-	-484	1	Ø.3
Co	241.7	Ø.2	6.Ø	-do-	-456	1	Ø.3

A standard for each element was run alongwith the test samples. Recovery experiments and replicate analysis were performed to determine the precision of the technique.

3.3 Histological Studies

Light Microscopy

(i) Fixation

Samples of normal peritoneum membrane were studied under light microscope. A small portion of the peritoneum tissue was removed immediately after slaughtering the experimental animal i.e. Bof. bubalis and immersed in 1Ø% formalin solution to preserve the tissue. The samples were minced into small fragments.

(ii) Dehydration

The minced pieces of the peritoneum tissue were dehydrated in a graded series of ethanol viz; 5Ø%, 7Ø%, 9Ø%, 95% and absolute alcohol with anhydrous sulphate, for 3-4 hours to 2 hours, respectively. After that, it was kept in

aniline oil overnight and was then transferred into xylene solution to clean the excess aniline.

(iii) Embedding:

The dehydrated pieces of the tissues were taken out from xylene solution and were transferred into a vial containing a mixture of paraffin wax in xylene and then to pure paraffin wax.

(iv) Sectioning

The pieces of the tissues embedded in paraffin wax were cut into the thin sections with the help of rotatory microtome. The sections were put on clean glass microslides and the slides were then placed in a warm current of water to remove the wax. Finally the sections were allowed to settle down on to the glass slides.

(v) Staining

The thin sections were stained with Hematoxylin and Eosine (HE) stain. The sections were again passed through the descending strength of ethanol prior to staining processes.

(vi) Mounting

After staining of the specimens, excess dyes were removed with alcohol washing and the sections were again

dehydrated in a graded series of alcohol upto the strength of absolute level. The sections were transferred to the solutions of a cleaning agent. A drop of mounting medium, canada balsam, was poured and the sections were covered with a coverslips and left to dry. After complete processing, photomicrograph of the prepared specimens were taken with different magnifications.

Scanning Electron Microscopy

Samples of peritoneum tissue were examined by the Scanning Electron Microscope (SEM). They were taken out immediately after slaughtering the experimental animal (Bof. bubalis) and were placed in a vial containing a few ml of cold Ø.1 M cacodylate - buffered 4% glutaraldehyde (pH 7.2) and minced into small fragments (1-5 mm in diameter). They were kept overnight at $4^{o}C$ and transferred to 1% OsO_4 in the same buffer for one hour in cold, Samples were dehydrated in a graded series of acetone and were dried on a Balzars Union Critical point drier using CO_2 as a critical point drying agent. The dried tissues were mounted on aluminium specimen stubs and coated with approximately 15 nanometer of gold palladium alloy on sputter coater (Polaron) and were observed under Philips 515 scanning electron microscope operating under 15-2Ø KV.

CHAPTER-4

RESULTS AND DISCUSSION

4.1 BIOPHYSICAL STUDIES

The membrane potential (Em or $\triangle \phi$) recorded in millivolts across the peritoneal membrane are plotted against the logarithm of average concentration, $\log(C_1+C_2)/2$, for various uni-univalent electrolytes in figure 2 and for various di-univalent and tri-univalent electrolytes in figure 3. The computed parameters α and β are given in table -1. The values of transference number of co-ions calculated from membrane potential by applying equations (5-7) are given in tables 2 and 3. The values of permselectivity for membrane-electrolyte systems have been worked out in tables 4 and 5. The thermodynamically effective fixed charge density of the membrane in contact with different 1:1, 2:1 and 3:1 electrolytes have been evaluated by the procedure of TMS (1,2) Kobatake and co-workers (3-5) and Tasaka et al. (6). The results are thus presented in tables 6 and 7.

The mobile species penetrate the membrane and various transport phenomena are induced into the system when two electrolyte solutions are separated by a membrane. In particular, a potential is generated which depends upon the charge on the membrane and its porosity. When the membrane pores are too wide, any charge does little to generate good potentials, but if the pores are narrow, a little charge on it gives a potential.

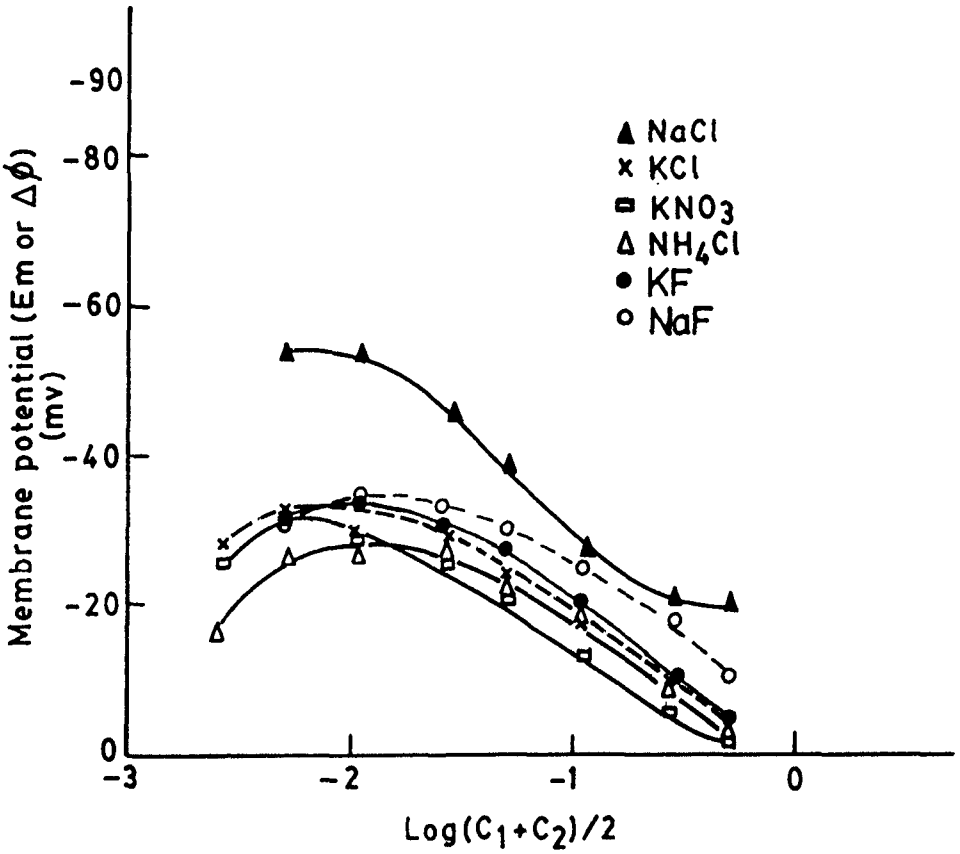


Fig.2: Plots of observed membrane potential ($\Delta\emptyset$ or Em) against log $(C_1 + C_2)/2$ for various electrolytes with peritoneal membrane.

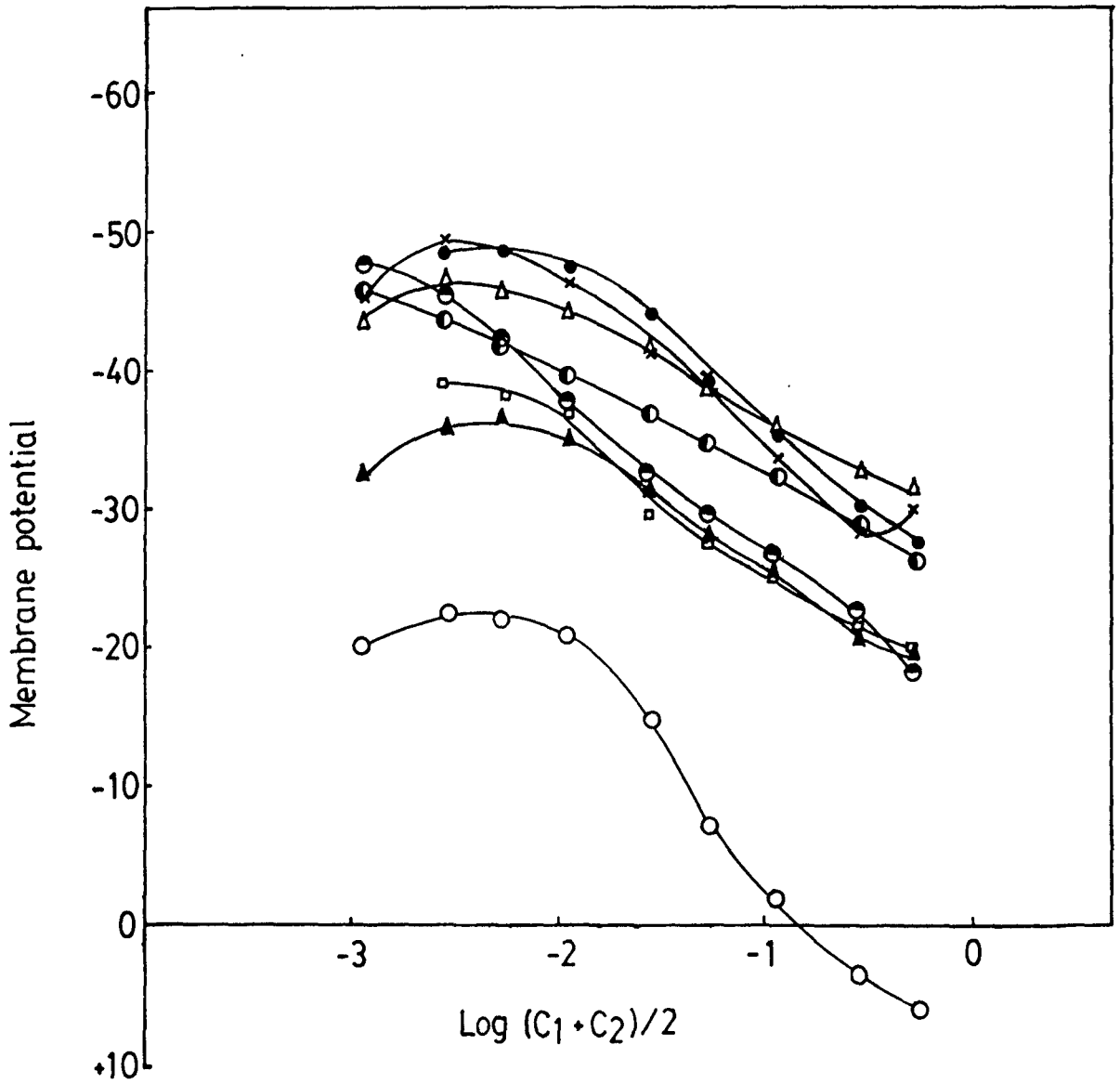


Fig. 3: Membrane potential (milli volts) versus log $(C_1+C_2)/2$ for different electrolyte solutions. Electrolytes: (×) $CaCl_2$; (▲) $MgCl_2$; (●) $ZnCl_2$; (△) $MnCl_2$; (⊖) $CuCl_2$; (○) Na_2SO_4; (◐) $CrCl_3$; (▫) $CoCl_2$.

The values of observed membrane potential are smaller when the membrane is used to separate concentrated electrolyte solutions, and the values are higher when it is separating dilute solutions. It is evident from the figures 2 and 3 that such behaviour of the membrane is peculiar to this system under investigation. Variation of membrane potential with different electrolyte concentration may be attributed to the change in selectivity character of the membrane for ions of electrolyte at different concentrations. In our experiment, it was observed that the potential could only be recorded when dilute side was taken as negative. Hence, the anion selective (positively charged) nature of the membrane under investigation unlike the cation selective behaviour in the case of synthetic membranes reported elsewhere (4-6,69,9Ø,129,134) is envisaged. It may, however, be noted that in our experimental studies for all the electrolytes below 2 x $1Ø^{-4}$ molar concentration no reasonable potential difference was observed. Such stepwise changes in membrane potential or selectivity character of the membrane electrolyte system may be explained in terms of structural changes produced in the electrical double layer at the interfaces.

The bi-ionic potentials can be easily recorded in the experiments on artificial membranes (35,36,92). However, in

cases of biomembrane Takeguchi and Nakagaki (194) reported that the bi-ionic potentials were not generally recorded. They attributed this behavioral difference between the artificial membranes and biomembranes on the basis of the fixed charges which are negative. Their work was done on the basement membrane (bovine lens capsule) which were found to be positively charge (194-196). We also found the peritoneum membrane as a positively charged membrane, and, therefore, it is possible to study bi-ionic potential on this biomembrane system.

In order to evaluate the different parameters by TMS equation (1) for the simple case of 1:1 electrolyte (22) and the membrane carrying a net charge ($\bar{X}$ =1), theoretical concentration potentials Em existing across the membrane were calculated as a function of C_2, keeping C_2/C_1 at constant 1Ø for different mobility ratios $\bar{u}/\bar{v}$ and were plotted in (fig. 4). The observed membrane potential values with different 1:1 electrolyte solutions were also plotted on the same graph. It is apparent in the graphic representation that the experimental curves shifted horizontally and ran parallel to one of the theoretical curves. The extent of this shift gave log $\bar{X}$, and the

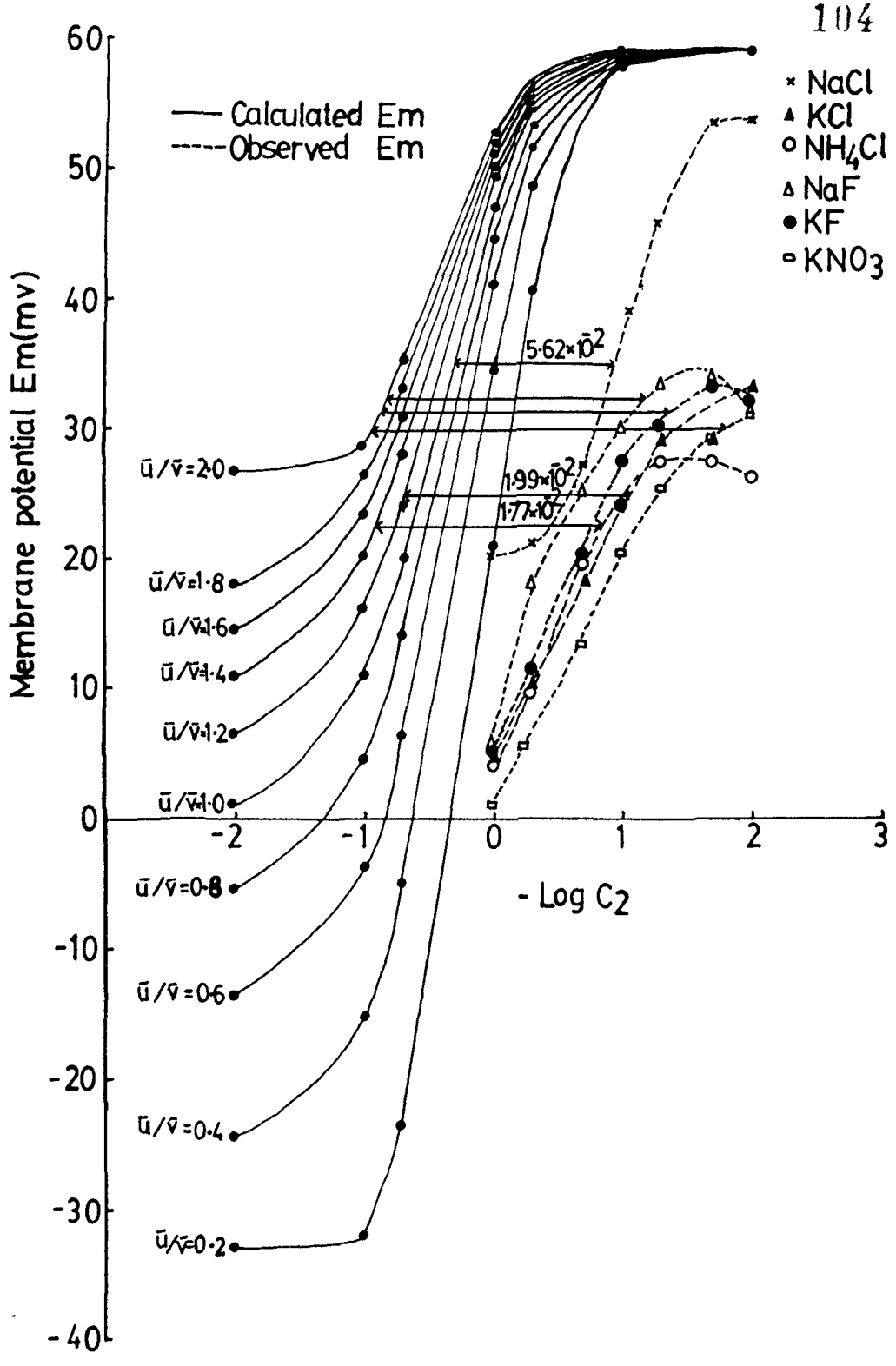


Fig. 4: Evaluation of the membrane charge density $\bar{X}$ and mobility ratio $\bar{u}/\bar{v}$. The smooth curves on the left are the theoretical concenteration potentials for a membrane ($\bar{X}=1$) at different mobility ratio $\bar{u}/\bar{v}$. The experimental values of Em for peritoneal membrane are shown by dotted lines.

parallel theoretical curves gave the value of $\bar{u} / \bar{v}$. The values for charge density ($\bar{X}$) derived in this way are given in table 6. It may be noted here that this method (TMS) has been generally used and widely accepted for evaluation of effective fixed charge density of a membrane.

The data of membrane potentials have been analyzed in terms of equations of Kobatake et al. (3) and the relevant empirical parameters, α, β and θ have been computed. The meaning of the charge density (θ) of the membrane, which appears in the electrical neutrality condition, here, is different from the original meaning of TMS. The θ is the product of the real charge density ($\bar{X}$) of the membrane and that of the interaction parameters ϕ, which is characteristics of a given pair of membrane and electrolyte ($\theta = \phi X$) (196). ϕ was discovered by Kobatake and Kamo (4) and was assumed to be independent from bulk concentration. Our treatment to the above facts are based on the assumptions that the mobile ion concentration equals to the analytical ion concentration while $\theta = \phi X$ is the same.

A variation in the values of reduced membrane potential ($\Delta\phi r$) with concentration, C_2 of various uni-univalent electrolytes (figs. 5,6) is employed to evaluate β from equation (18) and intercept of such plots, while α is

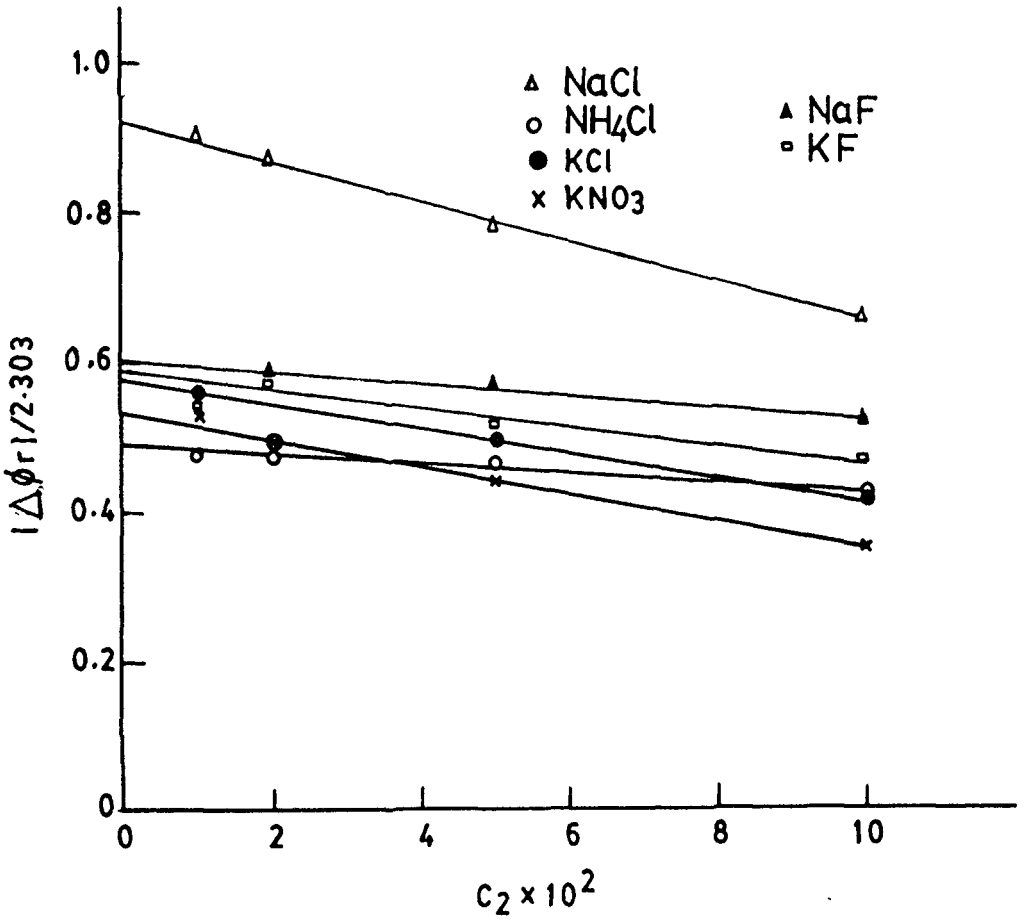


Fig.5 : Plots of $|\Delta\phi r|/2.303$ vs $C_2 \times 10^2$ for various electrolytes with peritoneum membrane.

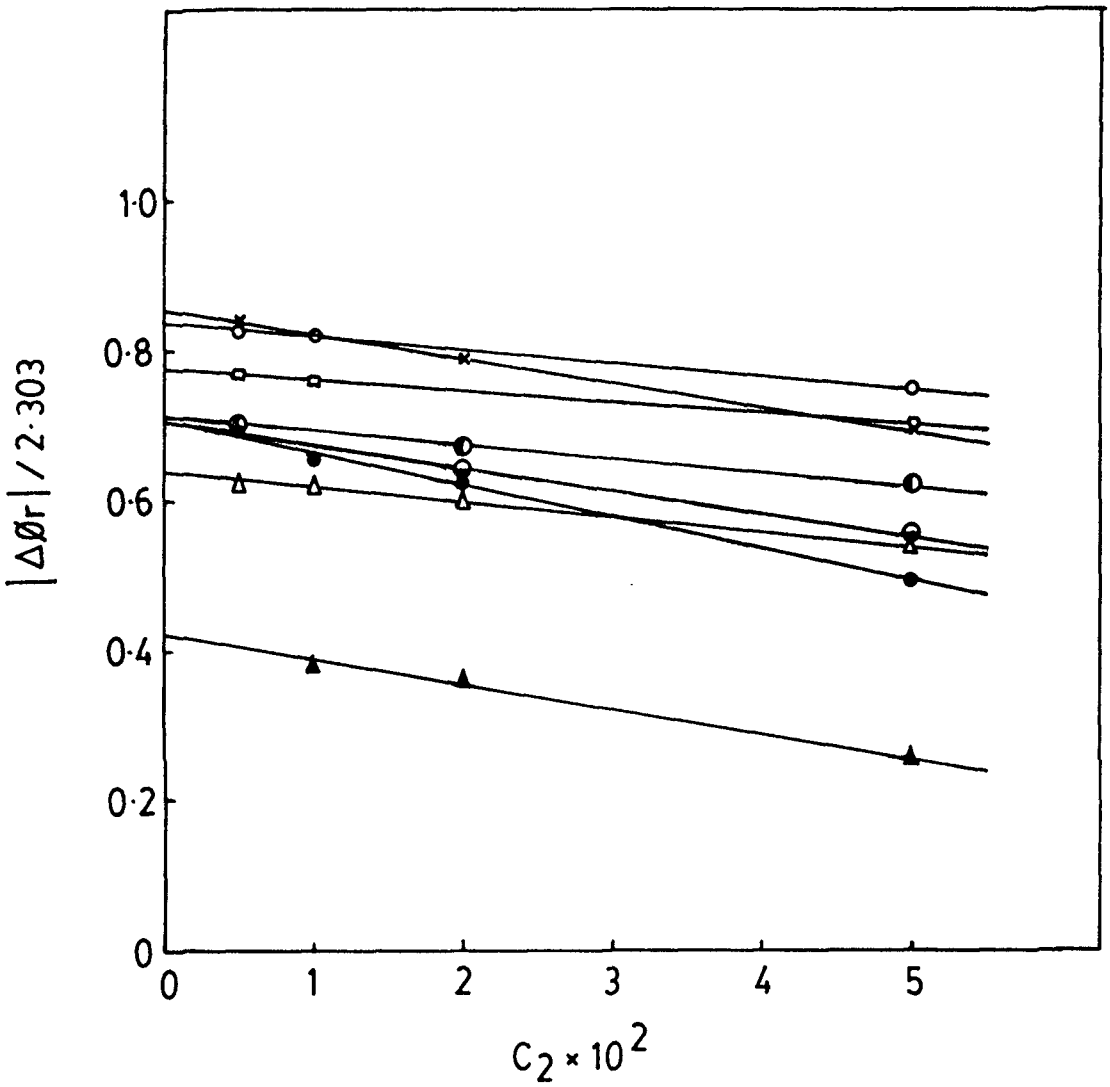


Fig. 6: Plots of reduced membrane potential $\Delta Ør / 2{\cdot}303$ versus C_2 for various electrolytes. Electrolytes: (×) $CaCl_2$; (Δ) $MgCl_2$; (○) $ZnCl_2$; (□) $MnCl_2$; (●) $CoCl_2$; (▲) Na_2SO_4; (⊖) $CuCl_2$; (◐) $CrCl_3$.

obtained from the intercept of the plot of 1/tapp (values of which are given in table-2,3) versus $1/C_2$ at a fixed value of γ (fig. 7,8). The value of α and β, are listed in table-1. By employing equation (18) and values of α and β, the values of charge density in dilute range (θd) are obtained. Similarly in the concentrated range the charge density (θc) has been obtained from the slope of the plots of figs.7 & 8 and equation (21), by substituting the values of α and β predetermined. These values of θd and θc are evaluated and listed in tables 6 and 7.

Kobatake et al. (3) equation $(\gamma - e^q) / (e^q - 1) = Z$ in which, $q = |\Delta\phi r| + (1+2\alpha) \ln \gamma / [1/\beta + (1-2\alpha)]$ and $Z = C_2/\alpha\beta\theta$ has been found to be applicable satisfactorily even to biological membranes as apparent from the plots of $\log (\gamma - e^q)/(e^q - 1)$ versus log Z (figs. 9 & 10). According to Kobatake et al. (3), if equation (16) is valid, the values of $(\gamma - e^q)/(e^q - 1)$ calculated from measured membrane potential ($\Delta\phi$) with the predetermined α, β and θ at the given value of γ must fall on a straight line which has a unit slope and passes the co-ordinate origin, when plotted against log Z. This behaviour must be valid irrespective of the value of γ and the kind of membrane electrolyte system. Figures 9 and 10 demonstrate that this theoretical

TABLE-1

THE DERIVED VALUES OF PARAMETERS α AND β FOR VARIOUS ELECTROLYTES WITH PERITONEUM MEMBRANE AT $\gamma = C_2/C_1 = 10$.

Electrolyte	Parameter	
	α	β
NaCl	0.459	1.086
KCl	0.474	1.724
NH_4Cl	0.487	2.040
KNO_3	0.445	1.886
NaF	0.545	1.652
KF	0.473	1.694
$CaCl_2$	0.574	1.170
$MgCl_2$	0.524	1.560
$ZnCl_2$	0.615	1.190
$MnCl_2$	0.677	1.280
$CoCl_2$	0.545	1.420
$CuCl_2$	0.565	1.420
$CrCl_3$	0.574	1.410
Na_2SO_4	0.565	2.380

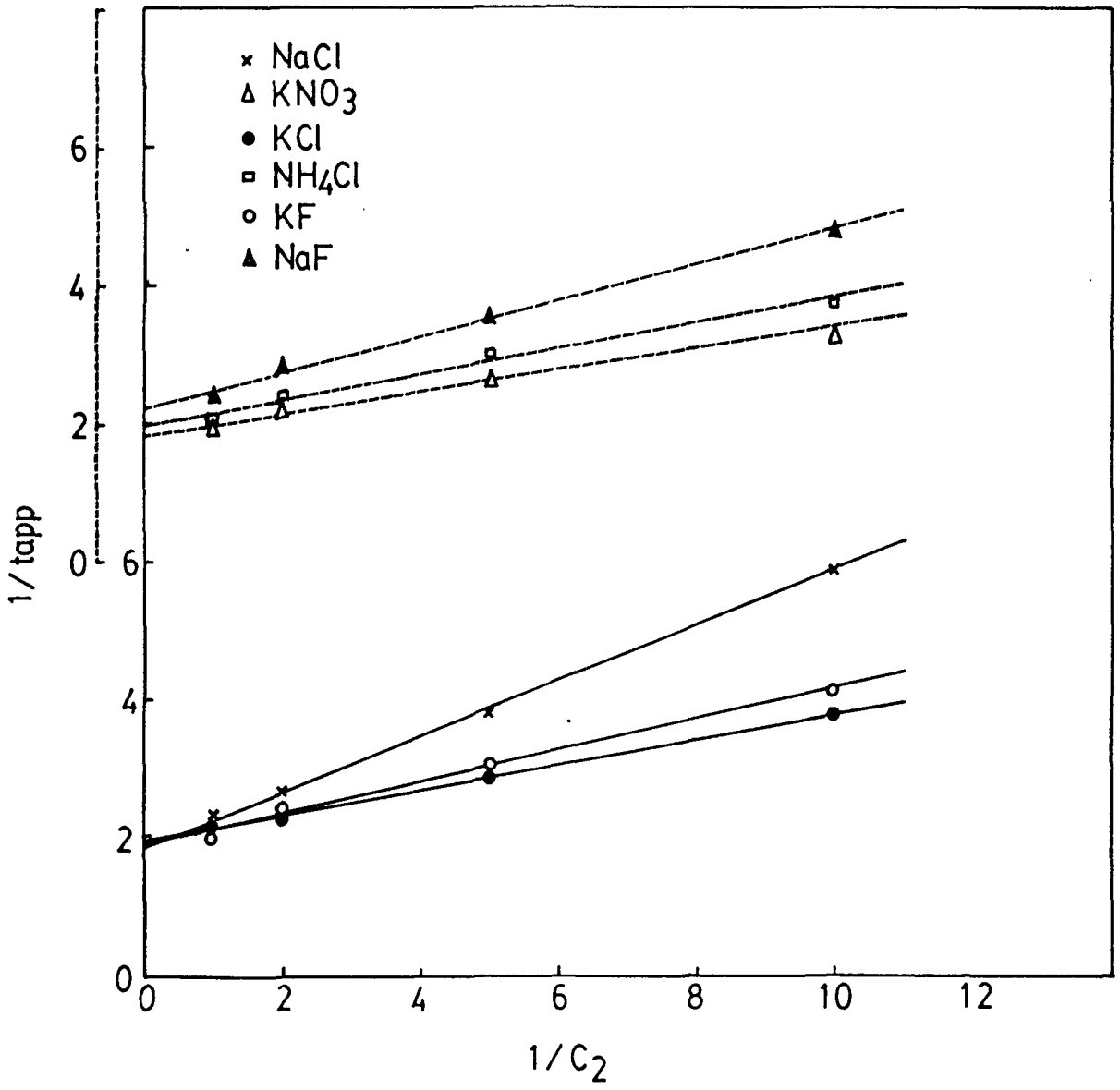


Fig.7: Plots of 1/tapp vs $1/C_2$ for various electrolytes with peritoneum membrane.

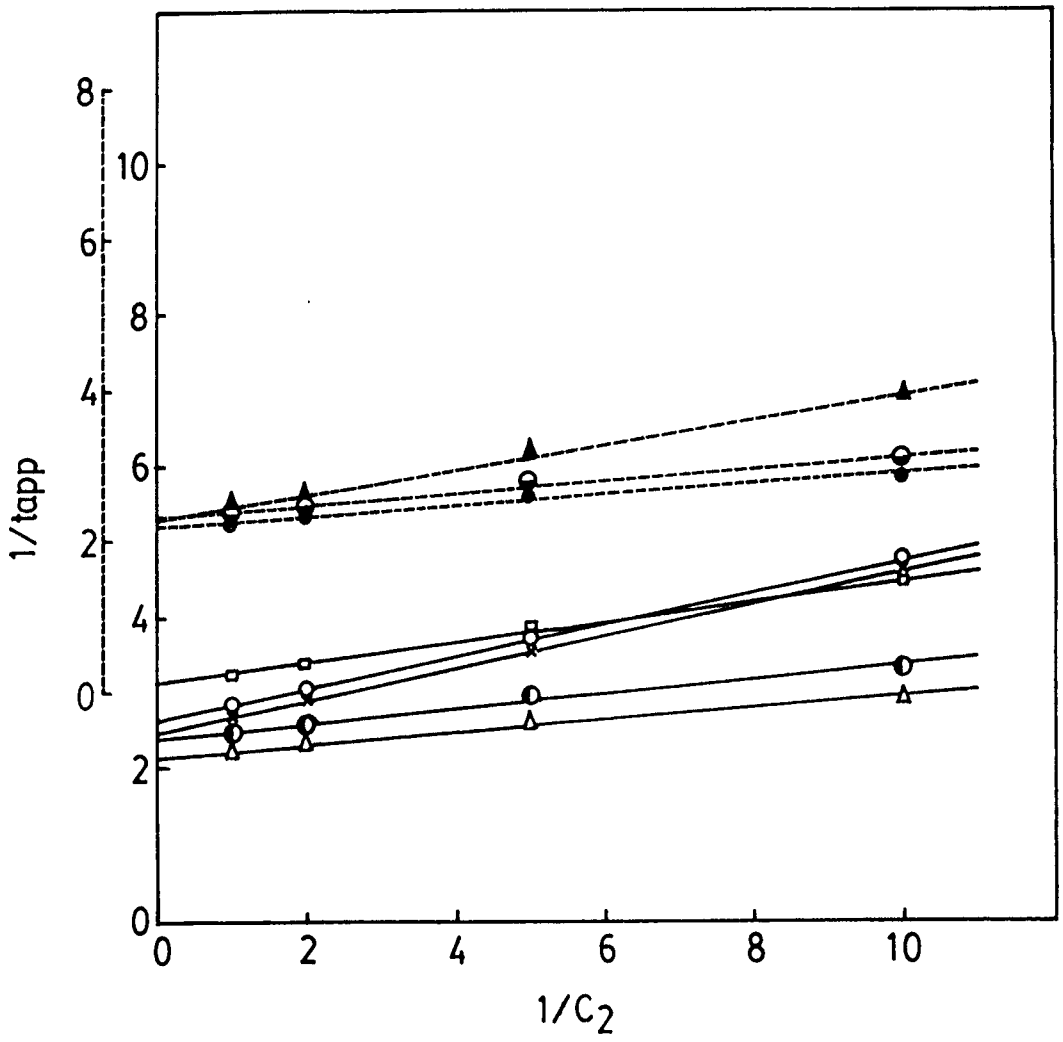


Fig. 8: Plots of inverse of transport numbers versus $1/C_2$ for various electrolyte solutions. Electrolytes: (×) $CaCl_2$; (△) $MgCl_2$; (○) $ZnCl_2$; (□) $MnCl_2$; (●) $CoCl_2$; (▲) Na_2SO_4; (⊖) $CuCl_2$; (◐) $CrCl_3$.

TABLE-2

THE TRANSPORT NUMBER (tapp) OF CO-ION DERIVED FROM THE OBSERVED MEMBRANE POTENTIAL AT VARIOUS ELECTROLYTE CONCENTRATION FOR PERITONEUM.

Electrolyte Concentration mole/liter	Transport Number					
	NaCl	KCl	NH_4Cl	KNO_3	NaF	KF
$10x10^{-1}/1x10^{-1}$	Ø.33	Ø.46	Ø.47	Ø.49	Ø.41	Ø.46
$5x10^{-1}/5x10^{-2}$	Ø.32	Ø.41	Ø.42	Ø.45	Ø.35	Ø.4Ø
$2x10^{-1}/2x10^{-2}$	Ø.27	Ø.35	Ø.33	Ø.38	Ø.29	Ø.33
$1x10^{-1}/1x10^{-2}$	Ø.17	Ø.3Ø	Ø.29	Ø.33	Ø.24	Ø.27
$5x10^{-2}/5x10^{-3}$	Ø.12	Ø.25	Ø.27	Ø.28	Ø.22	Ø.25
$2x10^{-2}/2x10^{-3}$	Ø.Ø5	Ø.26	Ø.28	Ø.25	Ø.21	Ø.22
$1x10^{-2}/1x10^{-3}$	Ø.Ø5	Ø.22	Ø.36	Ø.24	Ø.24	Ø.23
$5x10^{-3}/5x10^{-4}$	-	Ø.26	Ø.36	Ø.28	-	-
$2x10^{-3}/2x10^{-4}$	-	Ø.29	-	Ø.29	-	-

TABLE-3

TRANSPORT NUMBER (tapp) OF CO-IONS CALCULATED FROM THE MEMBRANE POTENTIALS FOR VARIOUS BI-UNIVALENT AND TRI-UNIVALENT ELECTROLYTES AT DIFFERENT CONCENTRATION.

Electrolyte Concentration mole/liter	Transport Number							
	$CaCl_2$	$MgCl_2$	$ZnCl_2$	$MnCl_2$	$CoCl_2$	$CuCl_2$	Na_2SO_4	$CrCl_3$
$10x10^{-1}/1x10^{-1}$	Ø.33	Ø.45	Ø.35	Ø.31	Ø.44	Ø.46	Ø.4Ø	Ø.42
$5x10^{-1}/5x10^{-2}$	Ø.35	Ø.42	Ø.33	Ø.3Ø	Ø.43	Ø.42	Ø.37	Ø.39
$2x10^{-1}/2x10^{-2}$	Ø.29	Ø.38	Ø.26	Ø.26	Ø.38	Ø.37	Ø.31	Ø.34
$1x10^{-1}/1x10^{-2}$	Ø.22	Ø.35	Ø.22	Ø.23	Ø.36	Ø.33	Ø.25	Ø.31
$5x10^{-2}/5x10^{-3}$	Ø.2Ø	Ø.31	Ø.17	Ø.2Ø	Ø.31	Ø.3Ø	Ø.17	Ø.29
$2x10^{-2}/2x10^{-3}$	Ø.15	Ø.27	Ø.13	Ø.17	Ø.27	Ø.25	Ø.1Ø	Ø.25
$1x10^{-2}/1x10^{-3}$	Ø.Ø6	Ø.25	Ø.12	Ø.15	Ø.24	Ø.19	Ø.Ø9	Ø.23
$5x10^{-3}/5x10^{-4}$	Ø.11	Ø.26	Ø.12	Ø.14	Ø.23	Ø.15	Ø.Ø8	Ø.2Ø
$2x10^{-3}/2x10^{-4}$	Ø.16	Ø.3Ø	Ø.16	Ø.17	-	Ø.13	Ø.11	Ø.17

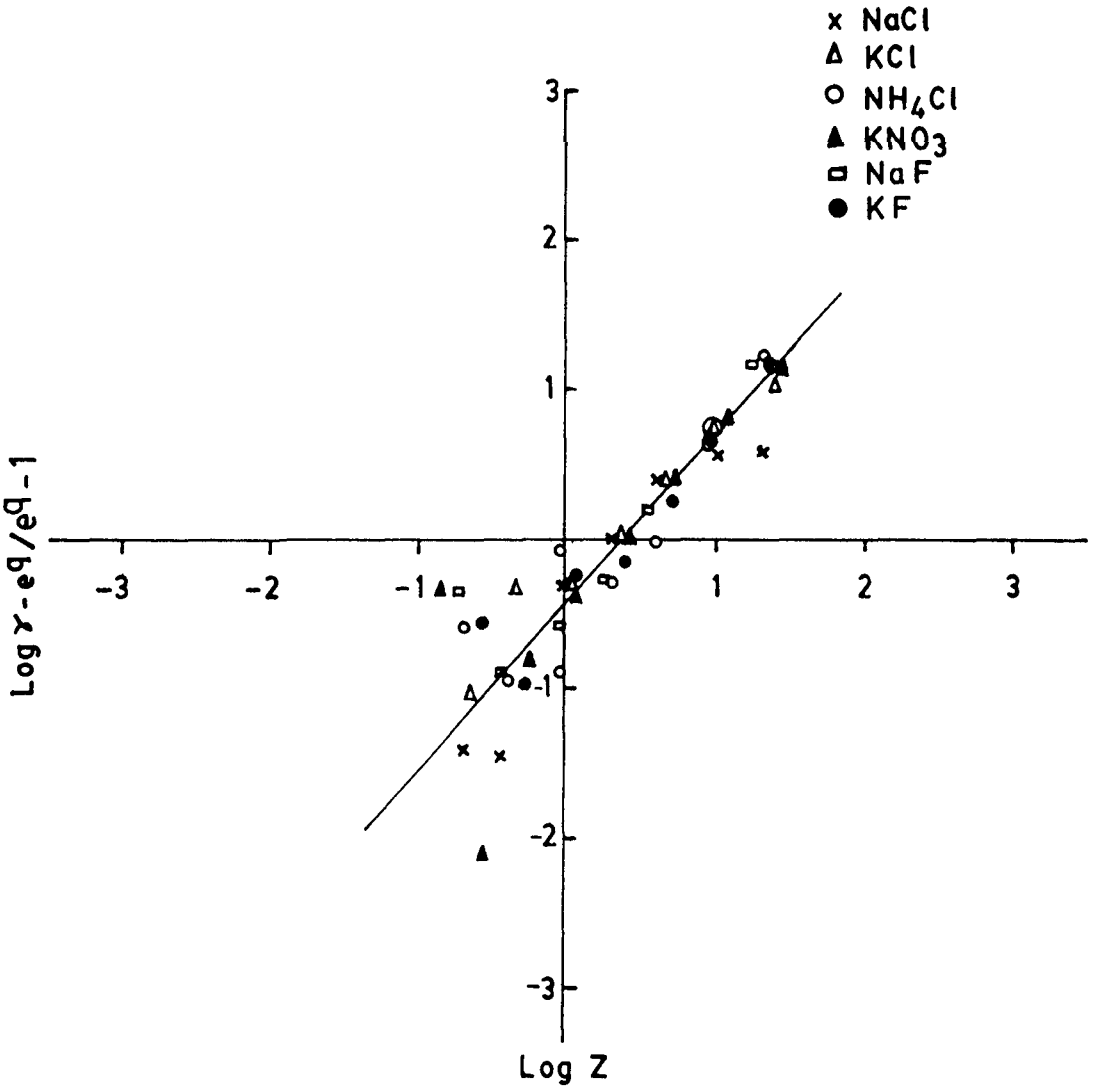
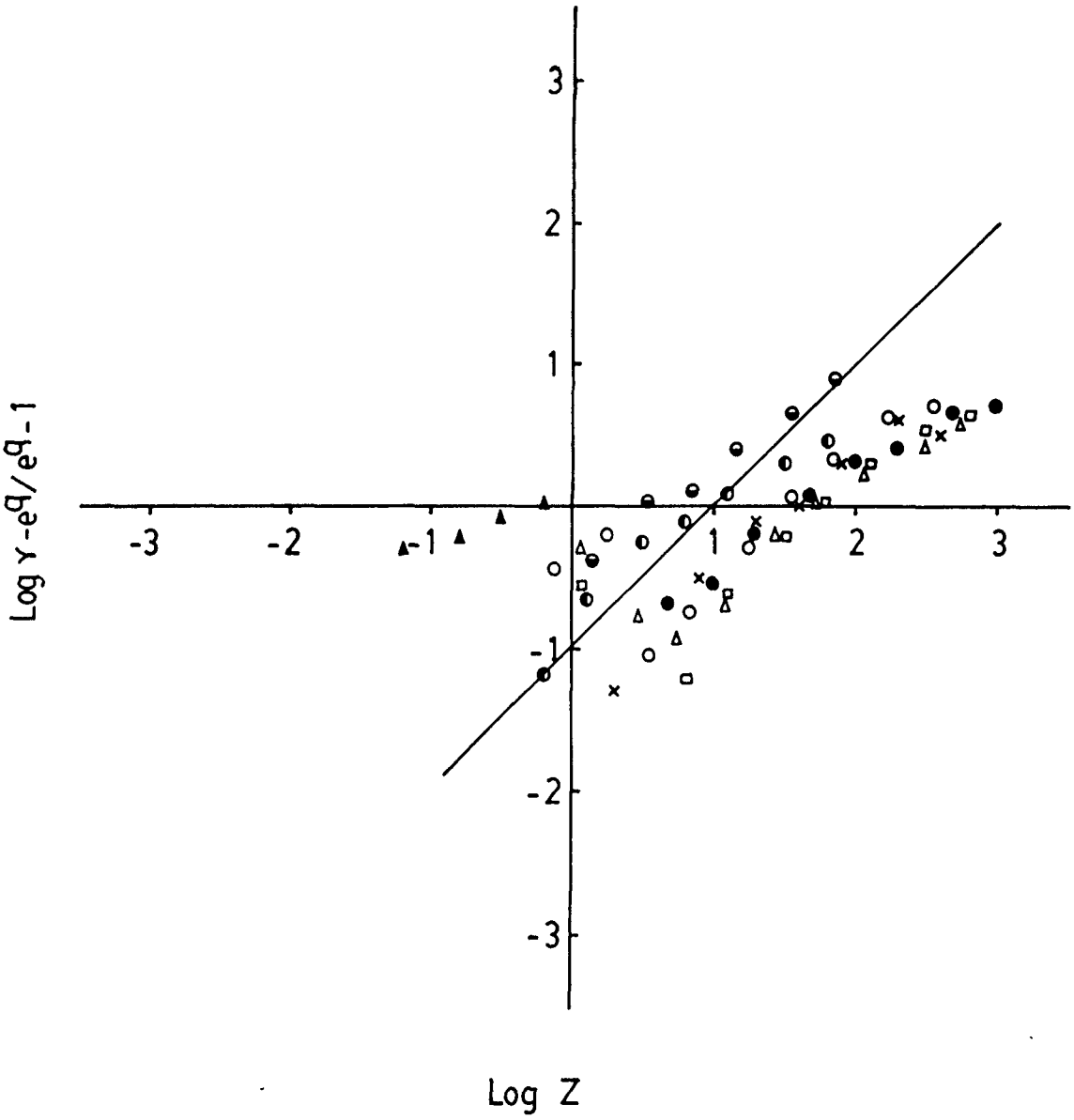


Fig.9: Plot of log $(\gamma - e^q)/(e^q - 1)$ vs log Z for various electrolytes with peritoneal membrane.



Fig·10: Plot of $\log\gamma - e^q/e^q - 1$ versus log Z for different electrolytes wit peritoneum membrane. Electrolytes: (×) $CaCl_2$; (▲) $MgCl_2$; (o) ZnC (□) $MnCl_2$; (●) $CoCl_2$; (▲) Na_2SO_4; (⊙) $CuCl_2$; (⊙) $CrCl_3$.

prediction from the membrane potential equation is borne out quite satisfactorily by our experimental results.

Charge density was also calculated by employing equation (25). On neglecting the last term at higher concentration, this equation (25) reduces to that of a straight line. The linear plots of 1/tapp versus $1/C_1$ (figs. 11,12) at a fixed value of γ support the applicability of the equation (25). The slope of such plots yield the values of charge density (ϕX) which are given in tables 6 and 7 for different uni, bi and tri-valent electrolytes, respectively.

The values of tapp obtained by relation (5-7) have been compared with those obtained by employing the relation (3Ø). In view of less than 2 % difference in the values of Tapp and tapp, both are considered to be essentially the same. The values of Permselectivity (Ps) of positively charged membrane (5) evaluated by using those of Tapp and α values, are listed in tables 4 and 5 for different electrolytes at different concentrations. The similarity in the pattern of graph (figs. 13,14) of Ps versus log $(C_1 + C_2)/2$ in the present case with those of others (5) support the applicability of these equations to even biological membranes.

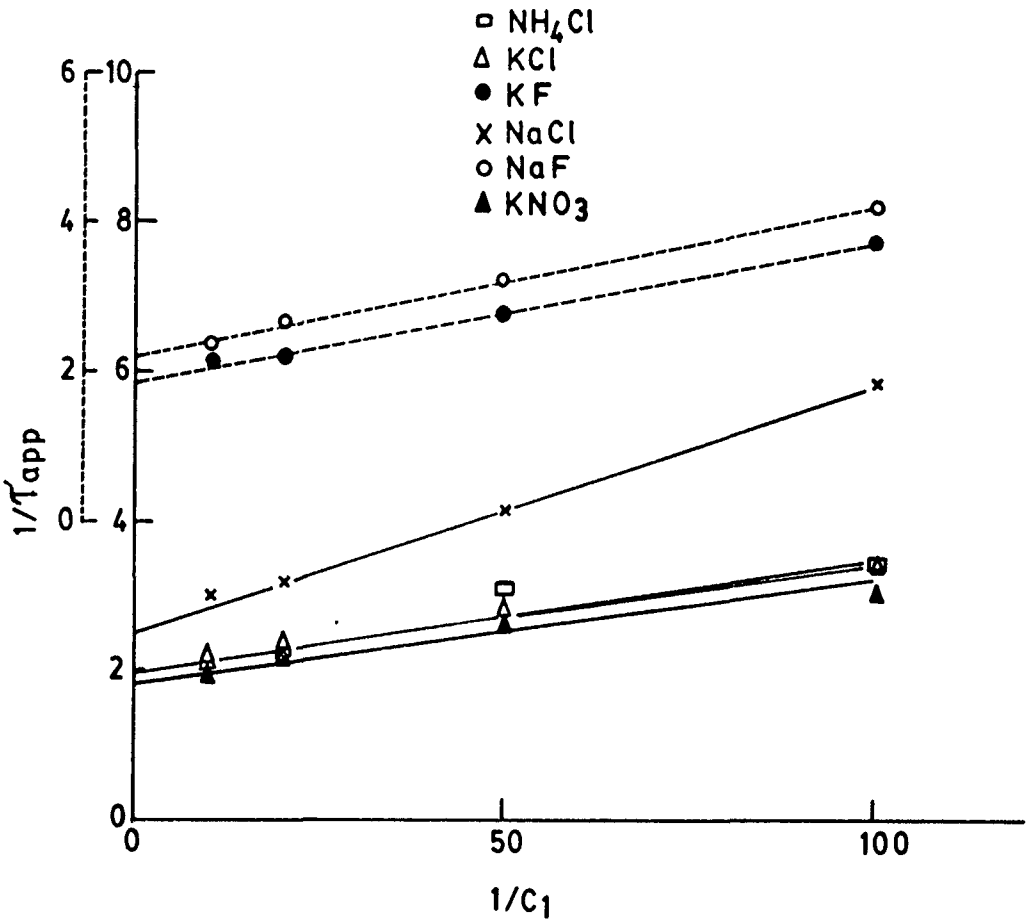


Fig.11: Plots of $1/\tau_{app}$ vs $(1/C_1)$ for various electrolytes with peritoneal membrane.

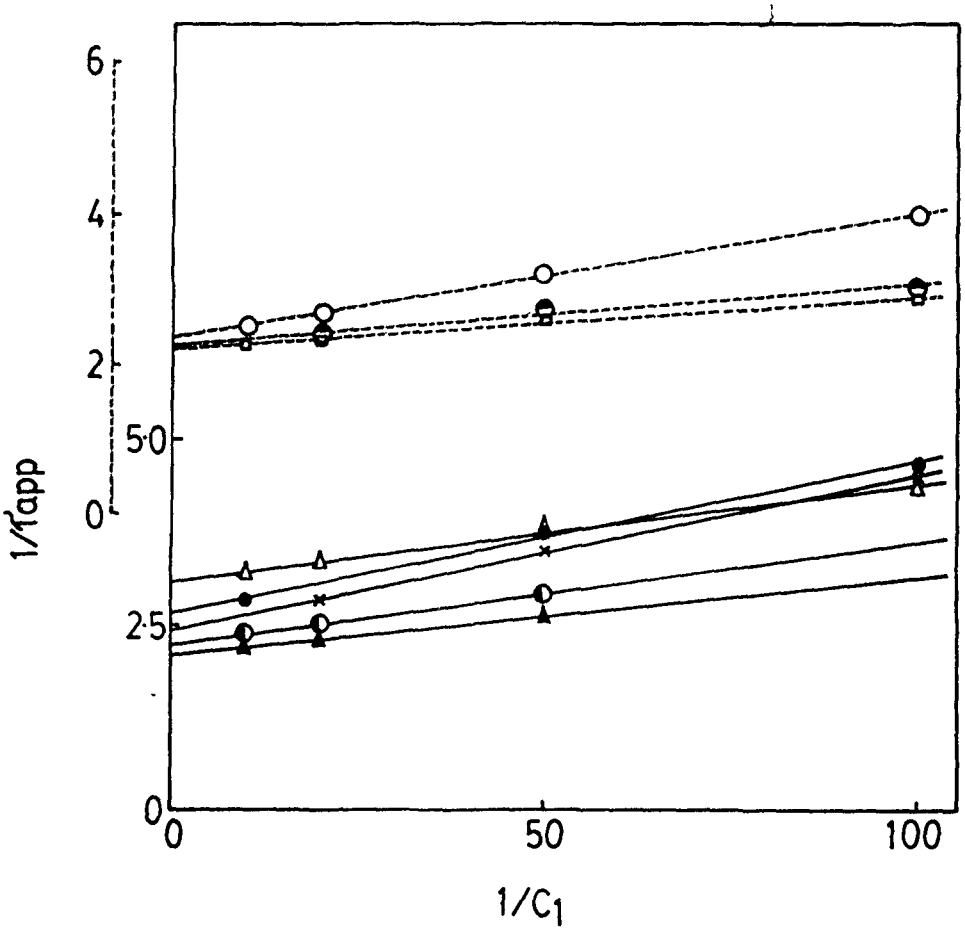


Fig. 12 : Plots of the inverse of transport number ($1/T_{app}$) versus $1/C_1$ for various electrolytes with peritoneum membrane. Electrolytes : (×) $CaCl_2$; (▲) $MgCl_2$; (●) $ZnCl_2$; (△) $MnCl_2$; (⊖) $CuCl_2$; (▫) $CoCl_2$; (○) Na_2SO_4 ; (◐) $CrCl_3$.

TABLE-4

VALUES OF PERMSELECTIVITY (Ps) OF MEMBRANE-ELECTROLYTE SYSTEMS FOR VARIOUS UNI-UNIVALENT ELECTROLYTES AT DIFFERENT CONCENTRATIONS.

Concentration mole/liter	Electrolyte					
	NaCl	KCl	NH_4Cl	KNO_3	NaF	KF
$10x10^{-1}/1x10^{-1}$	Ø.41	Ø.14	Ø.1Ø	Ø.13	Ø.1Ø	Ø.13
$5x10^{-1}/5x10^{-2}$	Ø.43	Ø.23	Ø.19	Ø.2Ø	Ø.23	Ø.25
$2x10^{-1}/2x10^{-2}$	Ø.52	Ø.36	Ø.36	Ø.34	Ø.35	Ø.39
$1x10^{-1}/1x10^{-2}$	Ø.7Ø	Ø.45	Ø.43	Ø.44	Ø.45	Ø.51
$5x10^{-2}/5x10^{-3}$	Ø.8Ø	Ø.53	Ø.49	Ø.52	Ø.5Ø	Ø.55
$2x10^{-2}/2x10^{-3}$	Ø.92	Ø.53	Ø.49	Ø.58	Ø.52	Ø.6Ø
$1x10^{-2}/1x10^{-3}$	Ø.92	Ø.6Ø	Ø.47	Ø.6Ø	Ø.46	Ø.57
$5x10^{-3}/5x10^{-4}$	-	Ø.52	Ø.3Ø	Ø.52	-	-
$2x10^{-3}/2x10^{-4}$	-	Ø.37	Ø.3Ø	Ø.5Ø	-	-

TABLE-5

THE VALUES OF PERMSELECTIVITY (Ps) OF MEMBRANE-ELECTROLYTE SYSTEMS FOR VARIOUS ELECTORATES AT DIFFERENT CONCENTRATIONS.

Concentration mole/liter	Electrolyte							
	$CaCl_2$	$MgCl_2$	$ZnCl_2$	$MnCl_2$	$CoCl_2$	$CuCl_2$	Na_2SO_4	$CrCl_3$
$10x10^{-1}/1x10^{-1}$	Ø.Ø6	Ø.Ø6	Ø.Ø4	Ø.Ø3	Ø.Ø3	Ø.Ø3	Ø.Ø5	Ø.Ø5
$5x10^{-1}/5x10^{-2}$	Ø.13	Ø.12	Ø.Ø8	Ø.Ø5	Ø.Ø5	Ø.Ø5	Ø.12	Ø.11
$2x10^{-1}/2x10^{-2}$	Ø.26	Ø.2Ø	Ø.25	Ø.15	Ø.15	Ø.15	Ø.25	Ø.22
$1x10^{-1}/1x10^{-2}$	Ø.42	Ø.26	Ø.35	Ø.23	Ø.2Ø	Ø.24	Ø.38	Ø.28
$5x10^{-2}/5x10^{-3}$	Ø.47	Ø.35	Ø.48	Ø.31	Ø.3Ø	Ø.3Ø	Ø.57	Ø.33
$2x10^{-2}/2x10^{-3}$	Ø.59	Ø.43	Ø.59	Ø.4Ø	Ø.3Ø	Ø.41	Ø.74	Ø.41
$1x10^{-2}/1x10^{-3}$	Ø.83	Ø.47	Ø.62	Ø.46	Ø.45	Ø.55	Ø.76	Ø.46
$5x10^{-3}/5x10^{-4}$	Ø.7Ø	Ø.45	Ø.62	Ø.49	Ø.47	Ø.64	Ø.79	Ø.52
$2x10^{-3}/2x10^{-4}$	Ø.57	Ø.37	Ø.51	Ø.4Ø	-	Ø.69	Ø.71	Ø.59

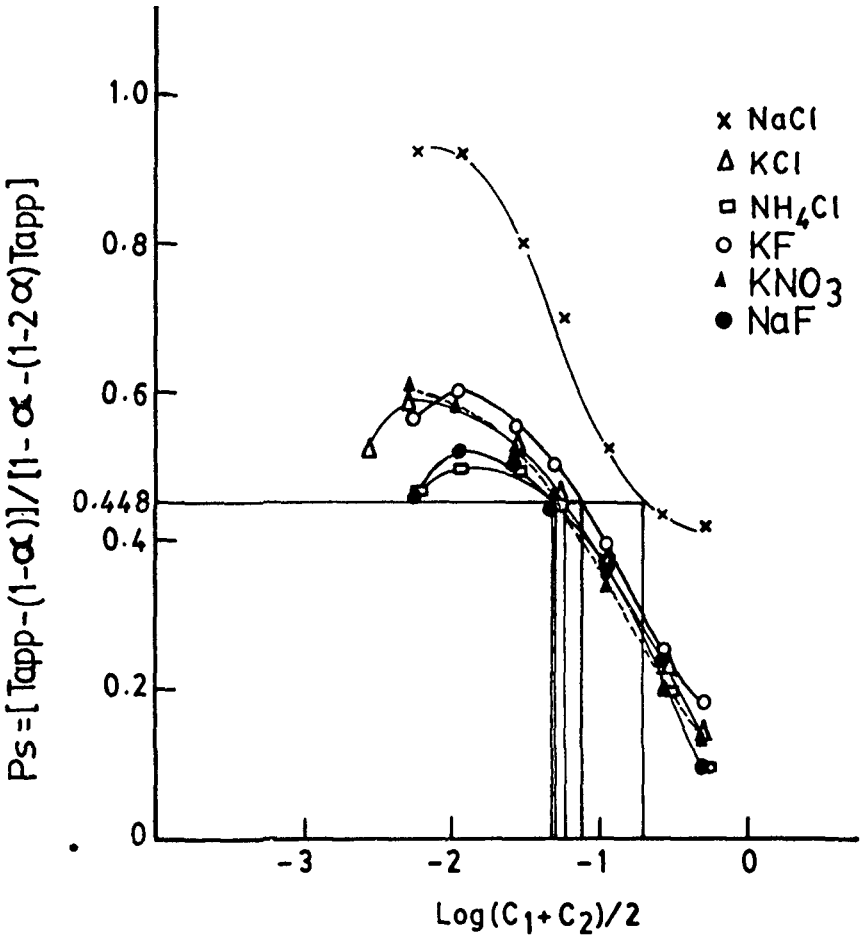


Fig.13: Plots of Ps vs $\log(C_1+C_2)/2$ for various electrolytes with peritoneal membrane.

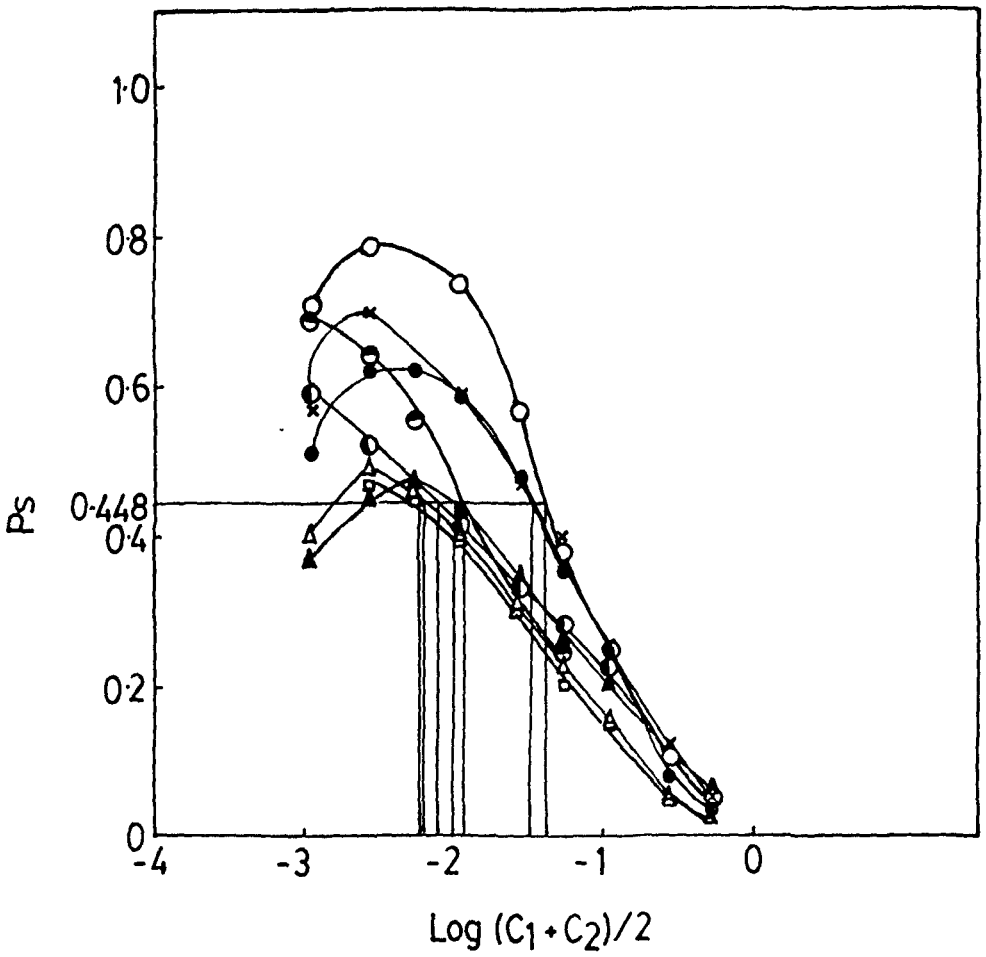


Fig. 14 : Plots of permselectivity (Ps) against log $(C_1 + C_2)/2$ for different electrolytes with peritoneum membrane. Electrolytes : (×) $CaCl_2$; (▲) $MgCl_2$; (●) $ZnCl_2$; (△) $MnCl_2$; (⊖) $CuCl_2$; (○) Na_2SO_4 ; (▫) $CoCl_2$; (◐) $CrCl_3$.

When the average concentration, $(C_1+C_2)/2 = C$ becomes equal to the fixed charge density, the values of ξ becomes unity, i.e. $\xi = C / \phi X = 1$. On substituting $\xi = 1$ into left hand side of equation (31) i.e. $Ps = (1 + 4\xi^2)^{-1/2}$, Ps turns out to be Ø.448. At this value of Ø.448, the corresponding concentration obtained from the plots of Ps vs log $(C_1 + C_2)/2$ (figs. 13,14) should be equal to the fixed charge density. In this way, the charge densities calculated with various electrolytes for peritoneal membrane are given in tables 6 and 7. Using the values of ϕX predetermined by the method developed by Kamo et al. (5), the Ps is replotted as a function of $(1+4\xi^2)^{-1/2}$, as shown in (figs. 15,16). An apparent straight line of slope close to unity is the theoretical line with the assumption that the values of ϕX are independent of salt concentration as seemingly found in case of NaCl, (fig. 15), while, those for the other electrolytes the values do not fall on a straight line, which shows that the charge density (ϕX) of the membrane is concentration dependent (figs.15,16).

We have also calculated the charge density, (ϕX) at higher concentrations of electrolytes using the equation (4Ø). The ϕX values from the plots (figs. 17,18) of Em vs $1/C_1$ for various electrolytes across buffalo peritoneum are given in the tables 6 and 7. An examination of these plots

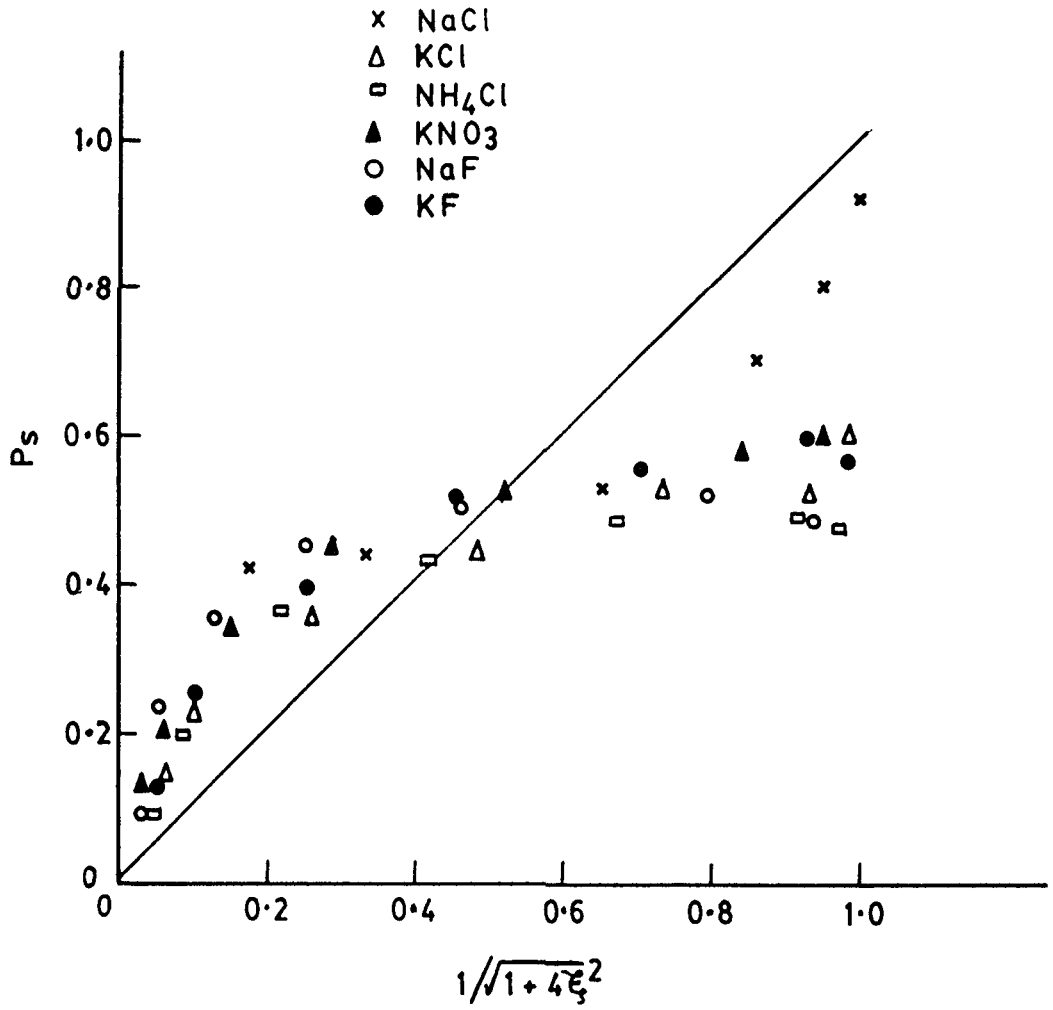


Fig.15: Plot of Ps vs $1/\sqrt{1+4\xi^2}$ for various electrolytes with peritoneal membrane.

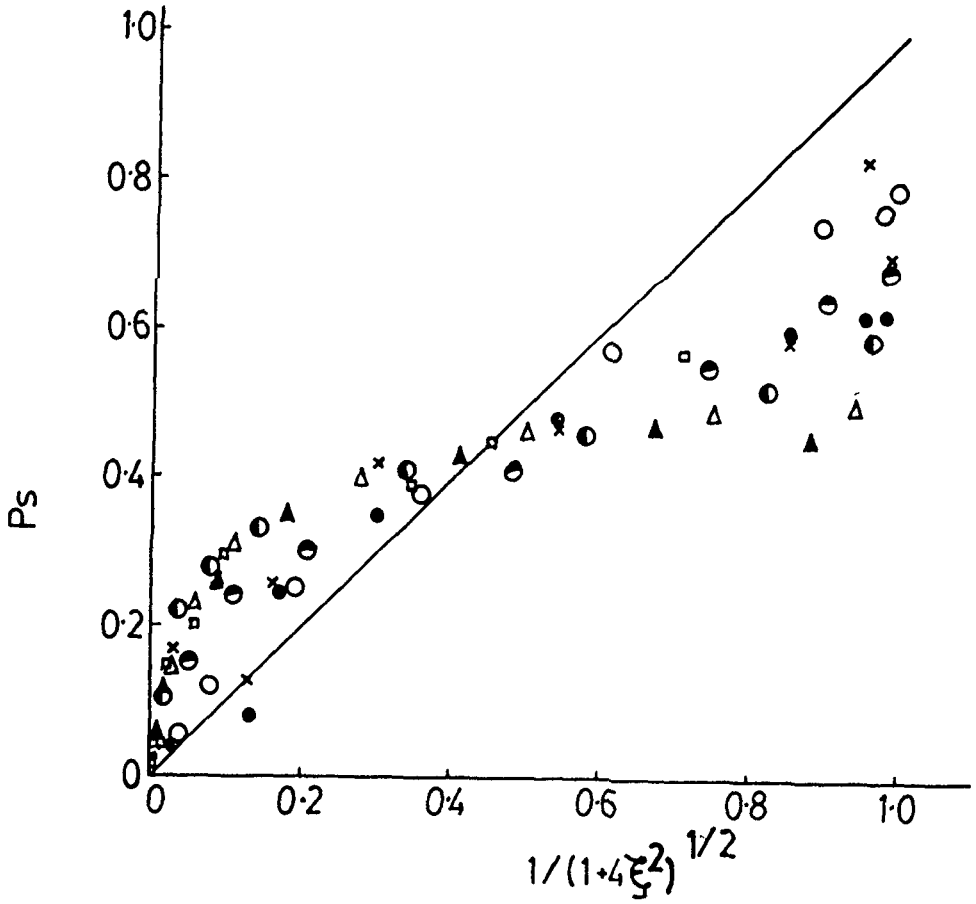


Fig. 16 : Plot of permselectivity (Ps) versus $1/(1+4\xi^2)^{1/2}$ for different electrolytes with peritoneum membrane. Electrolytes : (×) $CaCl_2$; (▲) $MgCl_2$; (●) $ZnCl_2$; (△) $MnCl_2$; (⊖) $CuCl_2$; (○) Na_2SO_4 ; (▫) $CoCl_2$; (◐) $CrCl_3$.

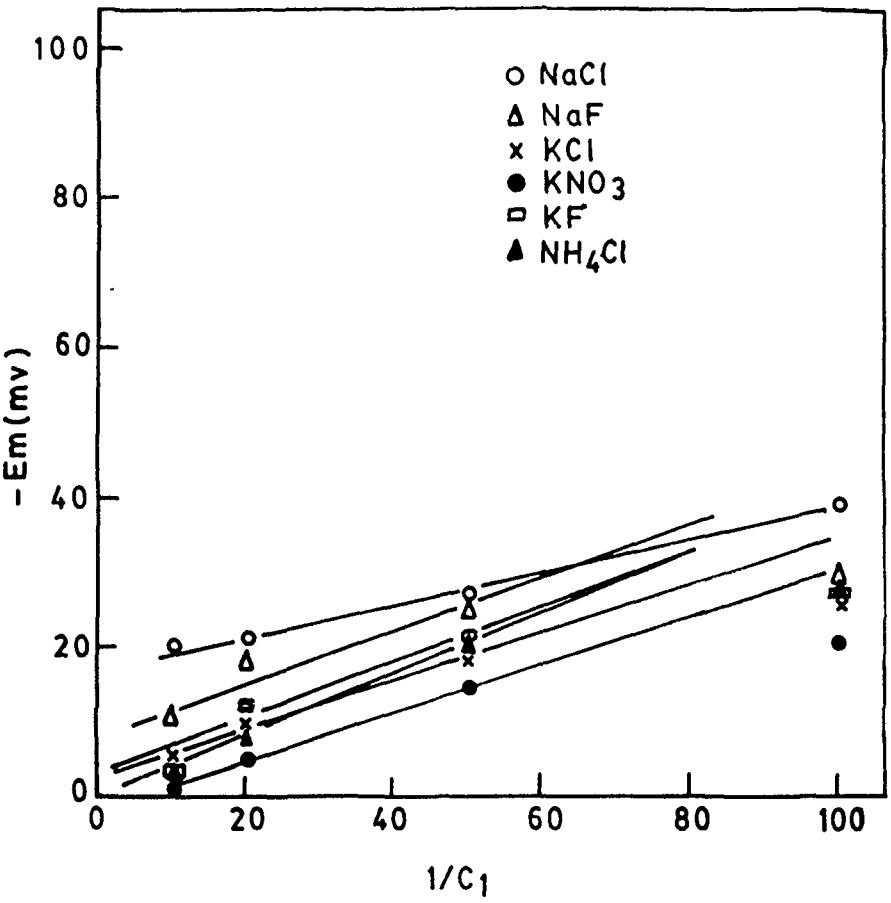


Fig.17: Plots of membrane potential (Em) vs $1/C_1$ for various electrolytes with peritoneal membrane.

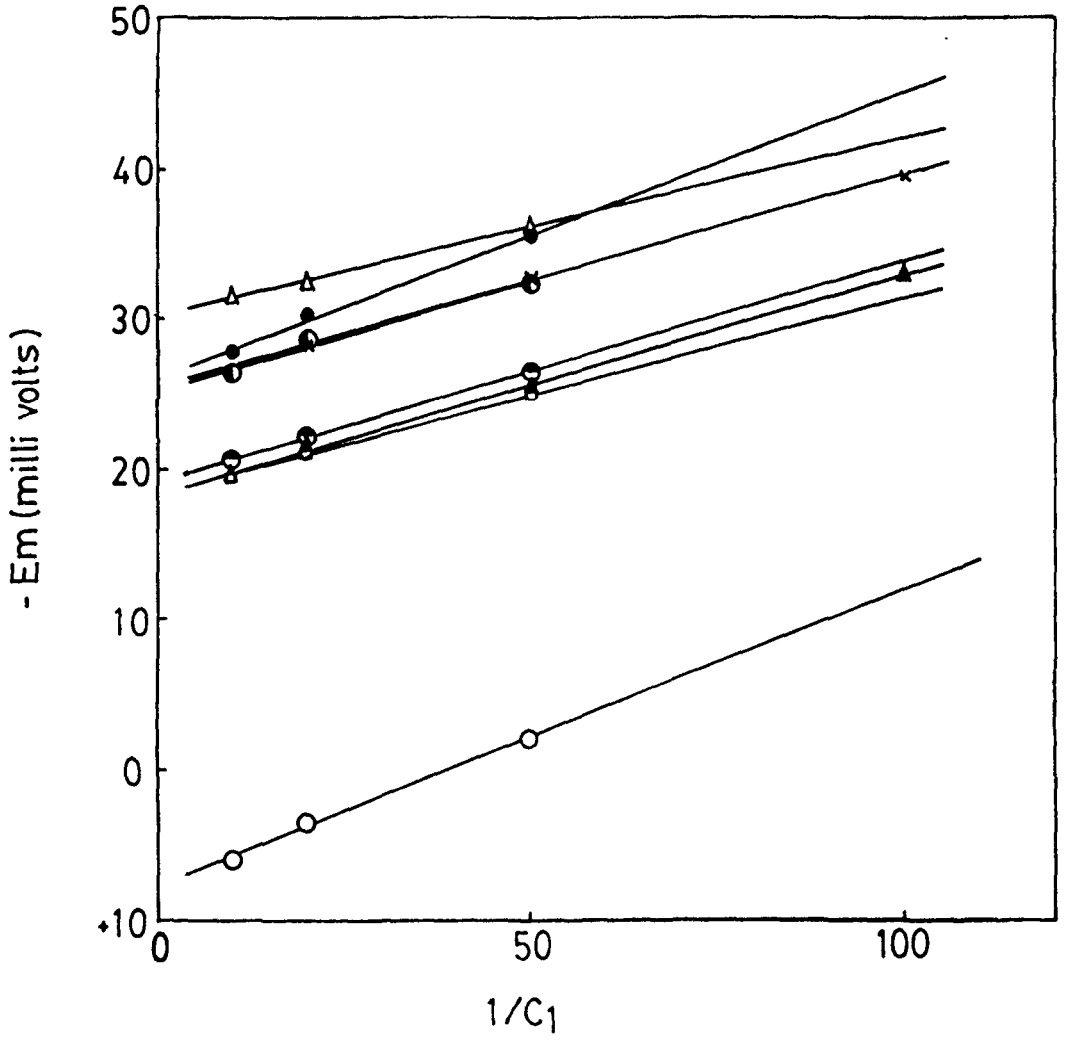


Fig.18 : Plots of membrane potential (Em) against $1/C_1$ for different electrolyte solutions with peritoneum membrane. Electrolytes: (×) $CaCl_2$; (▲) $MgCl_2$; (●) $ZnCl_2$; (△) $MnCl_2$; (⊖) $CuCl_2$; (▫) $CoCl_2$; (○) Na_2SO_4; (◐) $CrCl_3$.

reveals that the change in membrane potential is quite large with the change in concentration C_1.

Tables 2 and 3 give the results obtained by application of equations (5-7) for different electrolytes to the experimental data. From tables 2,3, the transport number values of co-ions (anion) decreases with decrease in electrolyte concentration. This can be explained by supposing that a potential barrier builds up progressively during diffusion of electrolyte solutions. This barrier, which is a double layers of ions at the immediate neighbouring of the anionic layer in the membrane, can hinder further diffusion. This result can also be explained by assuming the existence of positive charge on the membrane. This fixed charge will favour anion (co-ion) transport.

The TMS Theory (1,2), different theories of Kobatake et al. (3-5) and recently developed theory of Tasaka et al. (6) have been applied in our experimental system. We obtained the concentration range of ionogenic group within the membrane, also called as charge density, from the calculations used in different theories. It can be noted from the tables 6 and 7, that charge density of the membrane - electrolyte system are low. The values derived from

TABLE-6

CHARGE DENSITY (mole/dm^3) OF PERITONEAL MEMBRANE OBTAINED BY DIFFERENT METHODS AT $\gamma = C_2/C_1 = 10$, FOR DIFFERENT UNI-UNIVALENT ELECTROLYTES.

Theories	Electrolyte					
	NaCl	KCl	NH_4Cl	KNO_3	NaF	KF
TMS ($\bar{X}.10^2$)	5.6	1.4	1.8	0.2	1.0	0.6
$\bar{u}/\bar{v}$	1.0	1.2	1.4	2.0	2.0	2.0
Equation No(1)						
$\theta d \times 10^2$	17.6	18.2	32.4	17.1	25.5	25.1
Kobatake's method						
Equation No.(18)						
$\theta c \times 10^2$	10.2	5.5	6.3	4.4	6.3	5.2
Kobatake's method						
Equation No.(21)						
$\phi X \times 10^2$	19.0	6.0	5.0	5.3	5.0	7.9
Kobatake's						
Permselectivity method,						
Ps vs log $(C_1+C_2)/2$						
$\phi X \times 10^2$	7.6	4.1	4.1	4.4	4.7	5.7
Kamo et al. method						
Equation No.(25)						
$\phi X \times 10^2$	1.7	2.8	3.2	2.8	3.0	4.6
Tasaka's method						
Equation No.(40)						

TABLE-7

CHARGE DENSITY (mole/dm^3) OF PERITONEAL MEMBRANE OBTAINED BY DIFFERENT METHODS AT γ = 10 FOR VARIOUS 2:1 AND 3:1 ELECTROLYTES.

Theories	Electrolyte							
	$CaCl_2$	$MgCl_2$	$ZnCl_2$	$CuCl_2$	$MnCl_2$	$CoCl_2$	$CrCl_3$	Na_2SO_4
$\theta d \times 10^2$ Kobatake's method Equation No.(18)	12.4	14.2	18.6	8.8	12.9	16.4	15.0	2.5
$\theta c \times 10^2$ Kobatake's method Equation No.(21)	0.4	0.2	0.4	1.7	0.2	1.3	2.0	4.8
$\not{\theta} X \times 10^2$ Kobatake's Permselectivity method Ps vs log $(C_1+C_2)/2$	3.5	1.0	3.5	1.2	0.6	0.6	0.8	4.2
$\not{\theta} X \times 10^2$ Kamo et al. method Equation No.(25)	3.8	2.4	3.0	1.3	1.7	1.3	2.7	3.2
$\not{\theta} X \times 10^2$ Tasaka's method Equation No.(40)	1.1	1.3	1.6	1.3	1.0	1.2	1.2	1.7

different methods are almost the same. A little difference is formed which is attributed to the different graphical procedures adopted. The different methods applied herewith are valid for evaluation of the effective fixed charge density of the system. The values in the tables 6 and 7 also suggest that the charge density for uni-univalent electrolytes are greater than those for bi- uni and tri-univalent electrolytes. However, with 1:1 electrolytes solution, the membrane displays more anionic character than in the case of 2:1 and 3:1 electrolytes solutions. Thus, the concentration of ionogenic groups (fixed charge density) encountered in peritoneum membrane, despite their weak concentration, play an important role, particularly in electrochemical behaviour.

Biological membranes are selective. Using non-equilibrium thermodynamic treatment, it has been shown that ability of membrane to discriminate between solute and solvent can be adequately expressed in terms thermodynamic parameters (218). These parameters have been used for expressing selectivity of the natural and the artificial membranes. Permeation of a number of substances in the living system occurs through cell membranes with varying degree of restrictions. Like most of the biological membranes, the peritoneum membrane is not ideally semipermeable. It is a highly selective barrier with

restrictive properties comparable to those reported for continuous capillary beds. The study also revealed that peritoneal barrier in cat can be functionally characterized by equivalent small pore radius of approximately 6 nm, and about 1% of the peritoneal membrane filtration appears to be due to transcellular water passage (294).

Transport of neutral compounds can occur via intercellular pores or trancellularly. Permeability through intercellular pores usually is related to the molecular weight of the substance. Transcellular permeability usually depends more on lipid solubility and ionization. Intercellular pores present in the mesentery and parietal peritoneum provide a large area for absorption of small polar molecules and may help to explain the large percent absorption of low molecular weight molecules (32Ø).

On the basis of our findings, it can also be said that the electrochemical properties of peritoneum membrane, which is supposed to be composed of collagenous proteins rich in hydroxyproline and a carbohydrate, are quite stable in electrolyte solutions. It acts similar to synthetic ion exchanger membranes that bears relatively small quantity of positively fixed charges. Moreover, in contrast to certain membranes, such as, brush border membranes of small intestine and kidney, it is not known whether any

electrolytes are transported via a carrier mediated system across peritoneum or not. In any case, further investigation is needed to clarify the mechanism of the peritoneal transport of electrolytes.

Biological membranes, such as, frog's skin, gastric mucosa, toad bladder and goat urinary bladder, are considered to possess complex structure (37). Similarly, peritoneum which is a serous membrane, is also composed of several cell layers with protoplasmic liquids in between. This arrangement can be taken as multicompartmental structure. Anisotropic behaviour seems to be the most reasonable situation in this case. The chances of ultra filtration through peritoneum membrane, which consists of several layers, can therefore, be ruled out.

4.2 BIOCHEMICAL STUDIES

Water content

This study has been undertaken to characterize the biochemical components from buffalo peritoneum tissue. The water of peritoneum tissue has been estimated as 57% in weight of the fresh tissue. Normalization of this measurement has been performed maintaining the tissue samples for 1 hour at 20° and 60% humidity in preweighed vials prior to incubation in an oven at 50°C until constant weight gained.

Protein and lipid compositions

Protein and lipid components of peritoneum tissue were extracted. The protein content was found to be nearly 22% in weight of the dry tissue and about 77% of the peritoneum tissue dry weight is lipid. Among the lipid, the neutral lipid constitutes the major part. The composition of the total lipid fraction is given in table 8. Free fatty acid is the main component i.e. 59.6% of the total lipid.

The phospholipids composition as determined by the technique explained under 'Materials and Methods' is presented in table 9. Thin layer chromatography resolves the phospholipids present in the peritoneum tissue fragments into five spots. The phosphatidylethanolamine and phosphatidylcholine are the major phospholipids present in the total phospholipids components. The phosphatidylethanolamine content is somewhat greater than phosphatidylcholine (table-9) and together they account for about 79% of the phospholipids. Much of the remaining phospholipids is sphingomyelin, though small amount of phosphatidylserine and phosphatidylinositol were also observed in peritoneum tissue fragments.

The yields of peritoneum tissue fragments may be characterized by a lower protein content and higher lipid

TABLE-8

LIPID COMPOSITION OF BUFFALO (Bof. Bubalis) PERITONEUM TISSUE.

Lipid Class	Values
Phospholipids	15.3 ± Ø.8
Triglycerides	5.1 ± Ø.3
Free fatty acid	62.6 ± 1.6
Cholesterol	17.7 ± Ø.7

The values expressed as percentages ± SD

TABLE-9

PHOSPHOLIPID COMPOSITION OF PERITONEUM TISSUE

Component	% weight
Phosphatidylethanolamine	54.6 ± 1.3
Phosphatidylcholine	24.8 ± 1.Ø
Phosphatidylinositol	3.2 ± Ø.2
Phosphatidylserine	5.Ø ± Ø.2
Sphingomyelin	12.3 ± Ø.8

Values are expressed in terms of percentages ± SD.

content. From the tables 9 and 1Ø, it is conspicuous that peritoneum tissue has recorded higher total lipid alongwith its components, higher phospholipid fractions and component thereof compared to that of the finding on frog and toad skin and pericardial membrane in other studies (215,321). The phospholipid fractions varies differently and is lesser in amount than its neutral lipid components. The membrane permeability increases with the increase in the content of phosphatidylcholine and decreases with the increase in sphingomyelin content (322). The presence of high proportion of phosphatidylcholine and sphingomyelin (table-9), therefore, can be easily related to that of the permeability characteristics of peritoneum membrane.

ATP-ase activities

The data set out in table-10 represent the ATPase assay which has been performed in the total suspension after homogenization of the membrane tissue fragment. The findings suggest that of all the ATPases, the activity of Mg^{+2} ATPases is significantly higher and registers an activity of Ø.68 $\pm$ Ø.Ø6 expressed in terms of nanomoles Pi mg^{-1} protein min^{-1}. The next important ATPase, as evident from the data, is (Ca^{+2} - Mg^{+2}) ATPase which exhibits an activity of Ø.56 $\pm$ Ø.Ø3 n mole Pi. mg^{-1} protein. min^{-1}. (Na^{+} - K^{+}) ATPase is

TABLE-10

ATP-ase ACTIVITIES OF NORMAL BUFFALO PERITONEUM TISSUE EXPRESSED IN TERMS OF nano moles Pi/mg protein/minute

Enzyme	Activity
$(Ca^{+2} - Mg^{+2})$ - ATPase	Ø.56 ± Ø.Ø3 (5)
$(Na^{+} - K^{+})$ - ATPase	Ø.41 ± Ø.Ø5 (5)
Mg^{+2} - ATPase	Ø.68 ± Ø.Ø6 (5)

Values expressed in Mean ± SD.

Number in parenthesis are the number of experiments done separately.

next to follow, showing an activity of Ø.41 ± Ø.Ø5 unit. The activity of (Na^+ - K^+) ATPase is found to be the least among the ATPases assayed in peritoneum tissue fragments. The present results, therefore, reveal the presence of a complex enzyme system.

There is ample testimony as to the importance of phospholipids in the activity of plasma membrane associated enzymes, such as (Na^+ - K^+) ATPase and Mg^{+2} - ATPase (323). The phosphatidylserine and phosphatidylinositol appear to have specific roles in the function of (Na^+ - K^+) ATPase. The variations in the chain length and degree of unsaturation of phospholipid fatty acids have also been shown to affect the activity of this enzyme (323). The ATPase activity of peritoneum tissue fragments shows chiefly Mg^{+2} - ATPase. This is in agreement with other studies carried out on liver plasma membrane (323).

The acidic phosphatidylserine stimulates (Na^+ - K^+) ATPase activity and plays an important role in ionic transport (249). The lower quantity of phosphatidylserine may be linked with lower active Na^+ uptake. The presence of different ATPase activities can be related to different ion transport function of this membrane to possible ion pumps. (Na^+- K^+) ATPase, known to be involved at least directly in

ion transport, is present in the membrane fractions. But its contribution to the total ATPase activity is relatively minor. Besides (Ca^{+2} -Mg^{+2}) ATPase and (Na^{+}- K^{+}) ATPase, high level of Mg^{+2} ATPase activities was also detected. The high Mg^{+2} - ATPase and (Ca^{+2}- Mg^{+2}) ATPase activities are of considerable importance as the influence the transport across the biomembrane.

The Ca^{+2} is important in controlling the structure and adhesiveness of cell and also in regulating cellular activity and metabolism (266). The (Ca^{+2}- Mg^{+2}) ATPase is involved in the outward directed Ca^{+2} transport in human red blood cells (324). Thus (Ca^{+2}- Mg^{+2}) ATPases as present in the peritoneum tissue cells are contemplated to be involved in active uptake of Ca^{+2}.

However, there are interestingly additional problems which can be undertaken in future studies, such as, localization of different ATPase activities, further characterization and purification of different ATPase activities and examination of possible transport function in the tissues of peritoneum membranes etc.

Also, it is a well known fact that alteration in lipid composition and ATPase activities occur in different pathological conditions (233,249,325). It will, therefore,

be of interest to examine the effect of disease processes on the ATP-ase activities of the peritoneum.

Trace elements

Estimation of trace element was done by atomic absorption spectrophotometry. The trace elements estimated are given in table-11, it can be inferred that the Ca, Mg, Zn and Fe are the major trace elements present in the peritoneum. They together constitute nearly 80% of the total of 11 elements estimated in this study. Iron is present in high quantity, i.e. about 28% of the total elements analyzed. Next to follow are calcium and magnesium. The presence of Zn and Ni contents in appropriate amount can be related to the presence of different enzymes linked with the these metal ions. A notable feature was the high content of lead in buffalo peritoneum, which constituted 7.4% of the elements examined.

Trace elements are heterogenously distributed in biological tissues. The metal ions have non-homogenous distribution in different parts of the body, and this can be interpreted as an expression of functional difference at a given moment (278). Alteration in their concentration can lead to changes in different body functions. The normal values and pathological variation for a number of trace elements in a number of human diseases have been reported

TABLE-11

TRACE ELEMENTS ANALYSIS OF THE PERITONEUM TISSUE.

Element	Values in %
Ca	22.2 ± 8.7
Mg	17.6 ± 3.2
Zn	1Ø.5 ± 3.2
Co	3.7 ± Ø.5
Ni	6.9 ± 2.1
Fe	27.9 ± 4.2
Mn	Ø.5 ± Ø.1
Cu	1.7 ± Ø.1
Cd	1.3 ± Ø.6
Cr	Ø.1 ± Ø.ØØ4
Pb	7.4 ± 3.4

Values expressed in Mean ± SD.

earlier (326). The data shown in table-11 are useful and significant for further prospective elemental studies on peritoneum membrane under different disease states.

To sum up, it may be deduced that the data scientifically analyzed under the investigation are useful from view point of the chemical characterization exhibited by stabilization treatments of peritoneum membrane tissue.

HISTOLOGICAL STUDIES

Under light microscopy, the peritoneum tissues showed a simple epithelial lining, fibroblast cells and blood vessels. Loose connective tissue and loose areolar tissue (figs.19,20,23,24). Parallel running collagen fibers (fig.23) and a little collagen fibers were also distinctly visible (fig.25).

Normal buffalo peritoneum tissues were also observed under scanning electron microscope. Some specimen showed uneven epithelium (fig.27) and flat pavement epithelium (fig.28). Large number of pores were also discernible (fig.29) at higher magnification. Some specimens showed fiber bundles oriented in regular fashion (fig.29). Flat (squamous) cells and pores were also visible (fig.30).

Lentz (3Ø4) has described in detailed the ultrastructure of peritoneal mesothelial cells. Light microscopic studies reveal the presence of simple squamous epithelial cell lining in abundance in all the specimens. This indicates existence of squamous epithelial cell layer. Fibroblasts are generally responsible for the fibers formation. The exact nature of factors regulating fibroblastic activity are still controversial. Aalto et al. (327) showed that activated peritoneal macrophages release a soluble factor which stimulates the synthesis of collagen in fibroblasts. The higher abundance of blood vessels (fig. 19-26) may be related with those of passive diffusion of substances. Torres et al. (32Ø) suggested that portions covering mesentery represent the largest and most active area for transport since it is most richly supplied with the blood vessels. The substances placed in the peritoneal cavity tend to equilibrate with the blood in passive diffusion mechanism. The closely packed collagen interspersed with fibroblast may be understood to provide mechanical support to the membrane and may help in understanding the histophysiology of metabolites through peritoneum membrane. The transport studies across isolated mesentery suggest that the mesothelial cells don't contribute importantly to the transport of substances (328).

The scanning electron microscopic observation of regional structural differences in peritoneum membrane support the concept of regional functional specialization (329). The cilia which are the characteristics of all mesothelial cells are found to be absent. A large number of pores (fig.28&3Ø) are discernible at high magnification in the specimen studied. At lower magnification the epithelium unevenly distributed (fig.27) and fiber bundles (fig.29) can be easily seen. The microvilli and cell lines are the characteristics of normal mesothelium in the surface of thickened peritoneum (3Ø5,3Ø6) are not distinctly visible. Therefore, absorption through villous surface can be ruled out. The pores present at the surface of peritoneum tissue may be considered as the main transport pathways for the neutral compounds, small polar molecules as well as low molecular weight molecules. It has been investigated that the intercellular pores occupy Ø.6% of macroscopic area of the mesentery and Ø.2% of the area of parietal peritoneum. These intercellular pores can provide and may help to explain large absorption of different kind of molecules. Water, with 69% absorption, may move through intercellular pores as well as transcellularly (32Ø).

There are evidence that in certain disease processes like malaria, diabetes etc. the functioning of the affected

organs may get altered during physiological limitation or pathological conditions. Scanning electron microscopic studies on placenta and ovary showed that malarial infection might be responsible for several structural changes in the surface morphology of these organs (33Ø,331). Certain blocking agents are found to be the cause of secondary infection in peritoneum and can effective the histology of peritoneum by the formation of connective tissue and with the destruction of mesothelial cell membrane lining (3Ø6).

Fig.19 Normal peritoneum Specimen under light microscope shows the presence of simple squamous epithelial lining discontinuous at various places. Loose connective tissue, stroma with areolar tissue and fibroblasts cells.
Photomicrograph stained in Hematoxylin-Eosine x 40.

Fig.20 Normal peritoneum photomicrograph shows the presence loose areolar tissue, fibroblast cells and blood vessels. x 100; Stained with HE.

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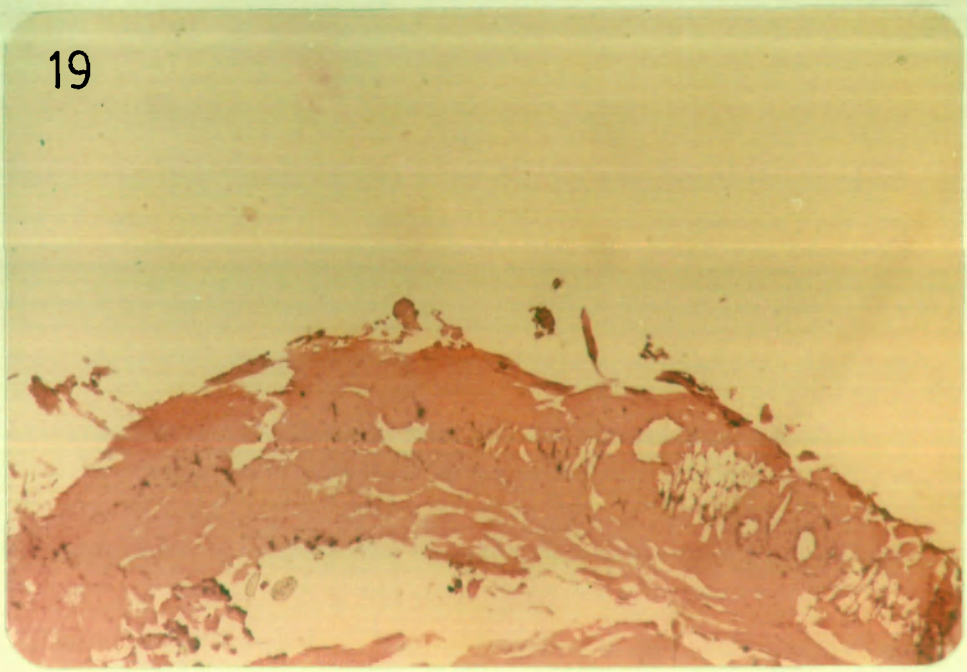

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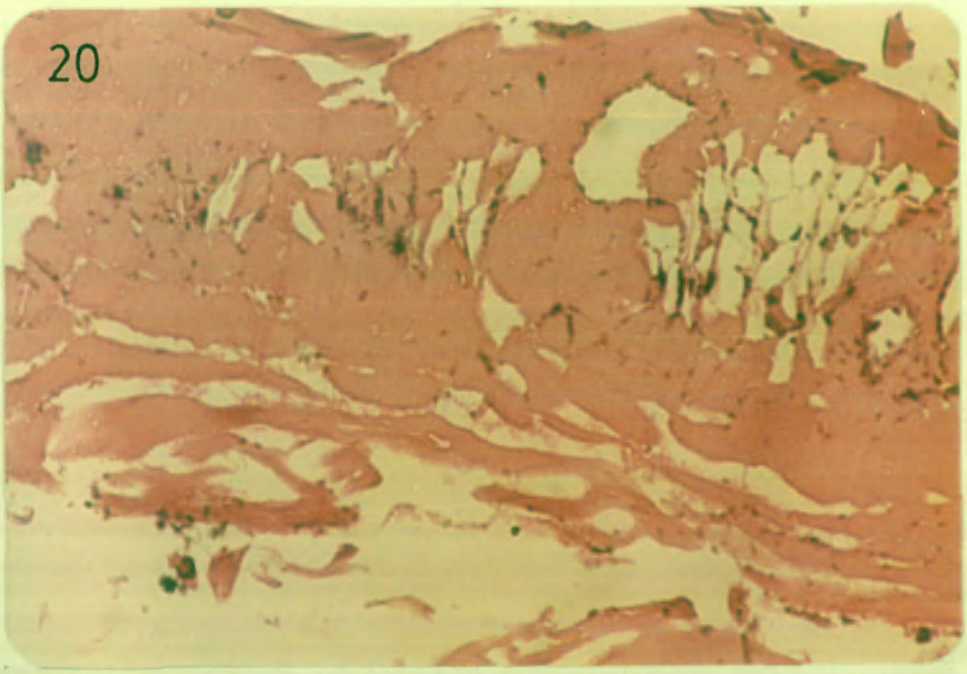
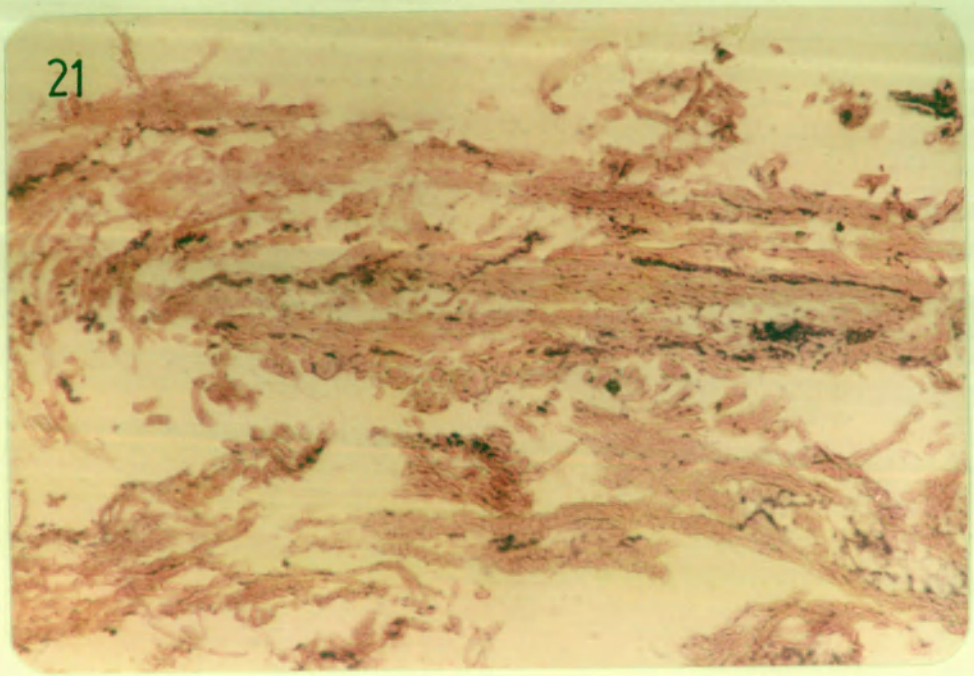


Fig.21 Normal peritoneum specimen seen under light microscope. Loose areolar tissue, simple squamous epithelium, fibroblasts and blood vessels are discernible. x 4Ø; HE stain.

Fig.22 Normal peritoneum specimen. Discrete areas of simple squamous epithelial lining, loosely arranged stroma, fibroblasts cells and blood vessels can be seen. x 1ØØ ; HE stain.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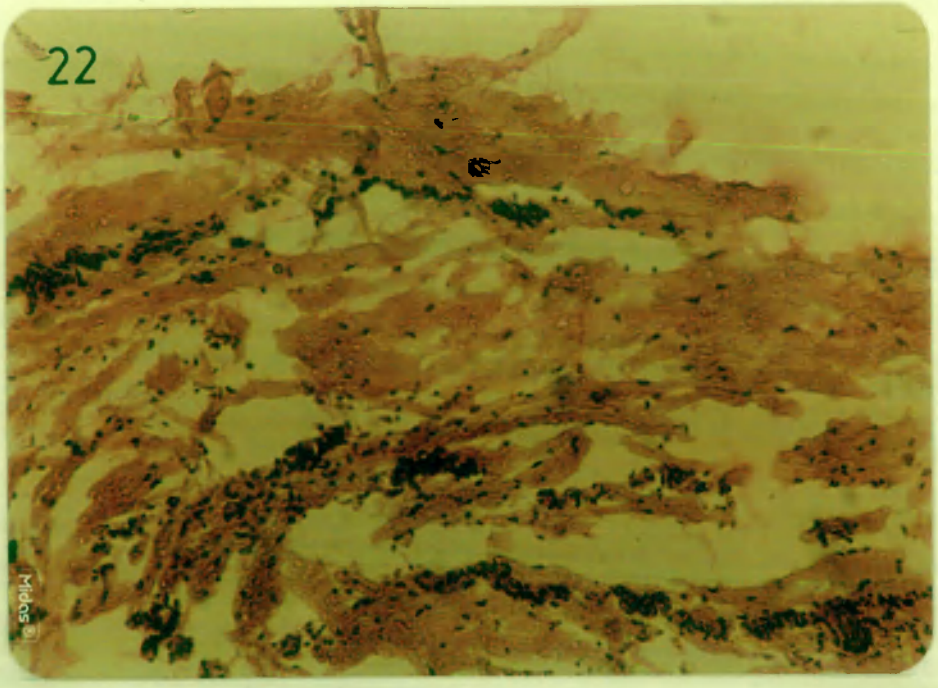
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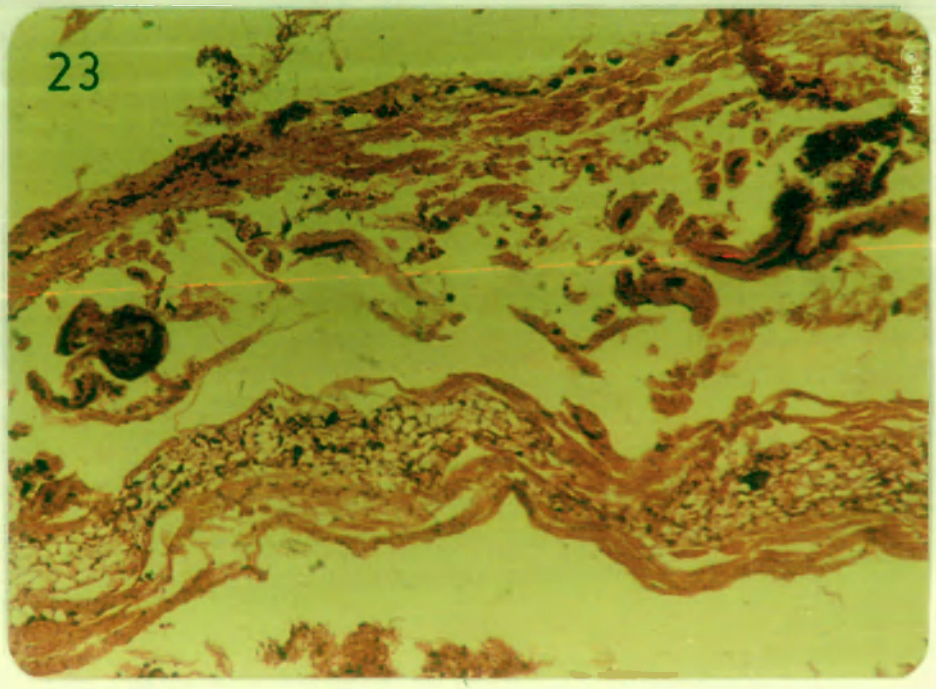
Fig.23 Normal peritoneum photomicrograph showing simple squamous epithelial lining broken at places, areolar tissue, fibroblast cells, blood vessels and parallel running collagen fibers. x 4Ø; HE stain.

Fig.24 Normal peritoneum specimen's photomicrograph showing discontinuous simple squamous epithelial lining. Loose areolar tissue, fibroblasts and blood vessels are also apparent. x 1ØØ; HE stain.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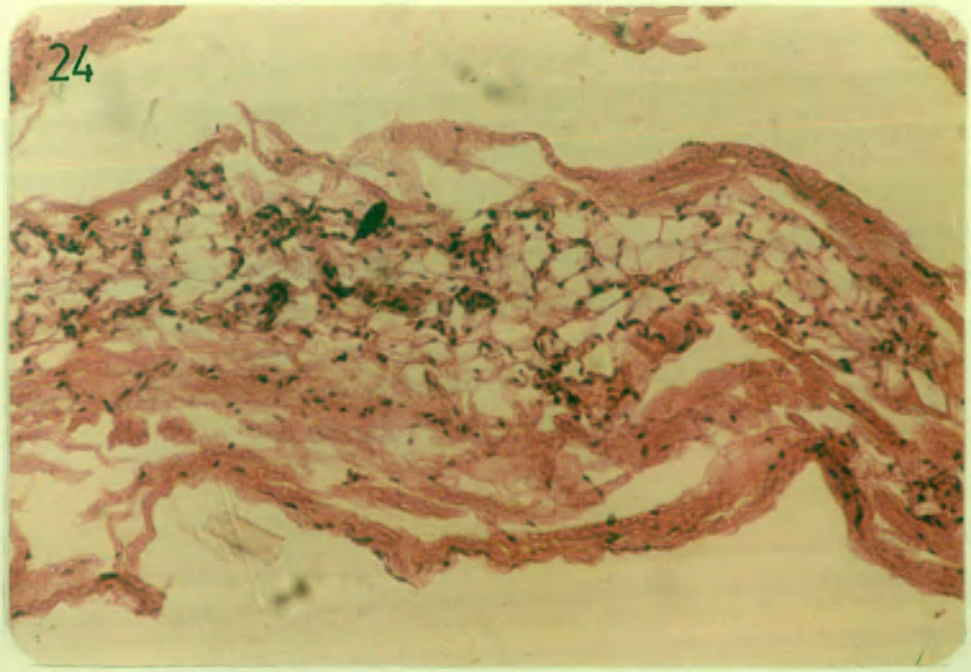
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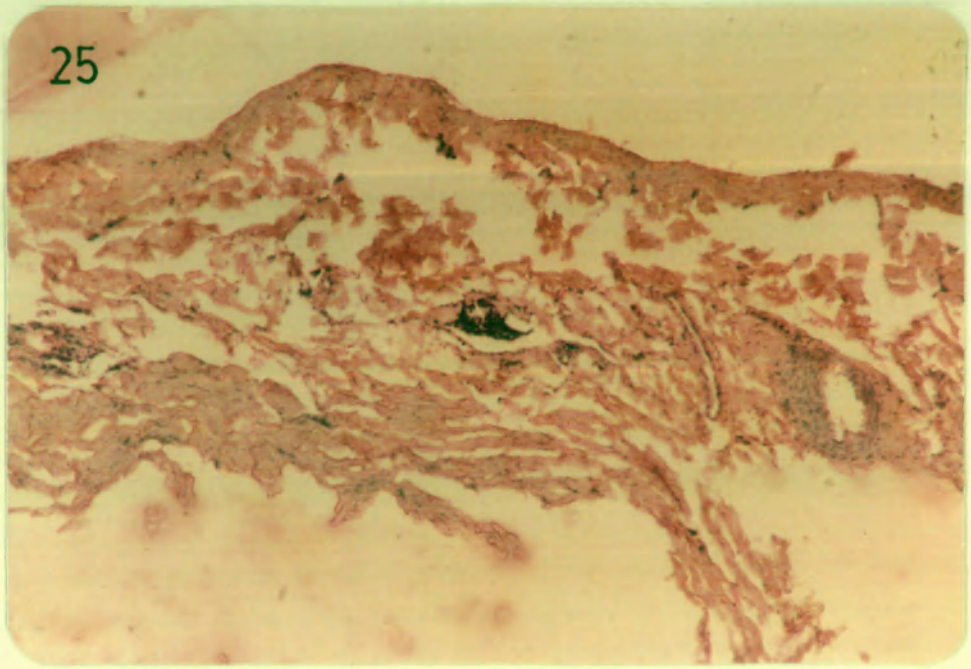
Fig.25 Normal peritoneum. Besides, simple squamous epithelial lining, loose areolar connective tissue, fibroblasts, blood vessels, a very little collagen fibers are observable. x 40; stained with Hematoxylin-Eosine.

Fig.26 Normal peritoneum specimen at high magnification. i.e. x 100; HE stain. Shows the same feature as seen in fig.25.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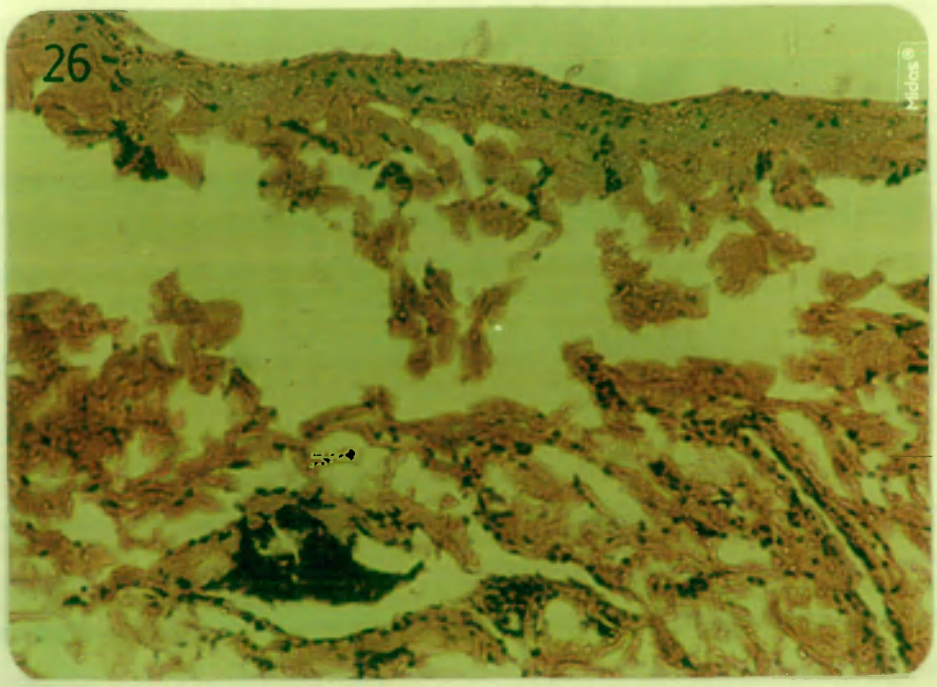
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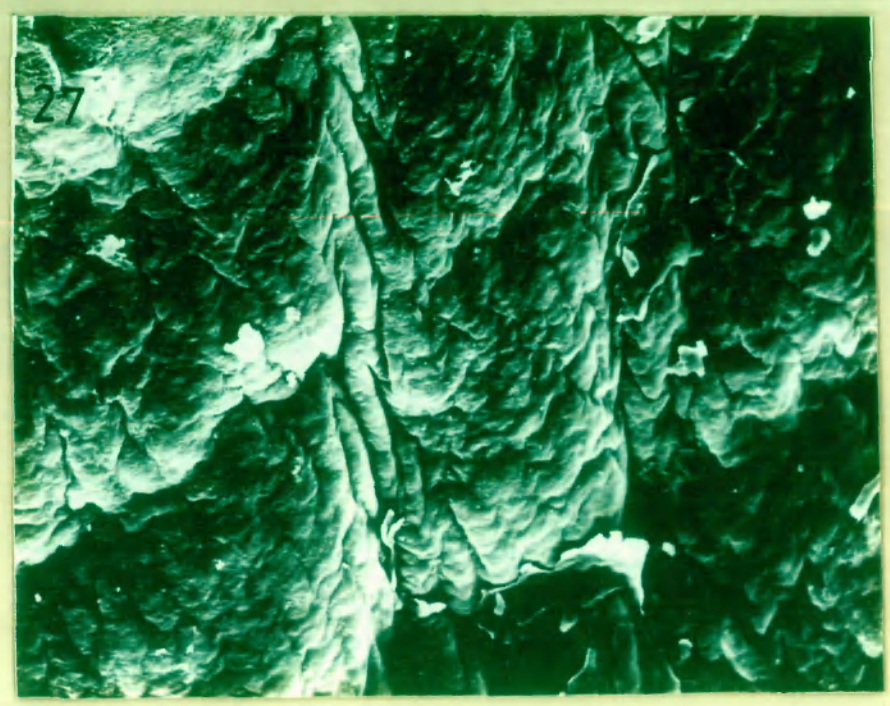
Fig.27 Scanning electron photomicrograph specimen showing surface fine structure of a part of the peritoneum. The uneven epithelium and some excrescences can be easily seen. Magnification 156x.

Fig.28 Surface fine structure of the peritoneum showing flat pavement epithelium. The large number of pores discernible in this scanning electron photomicrograph. x 155Ø.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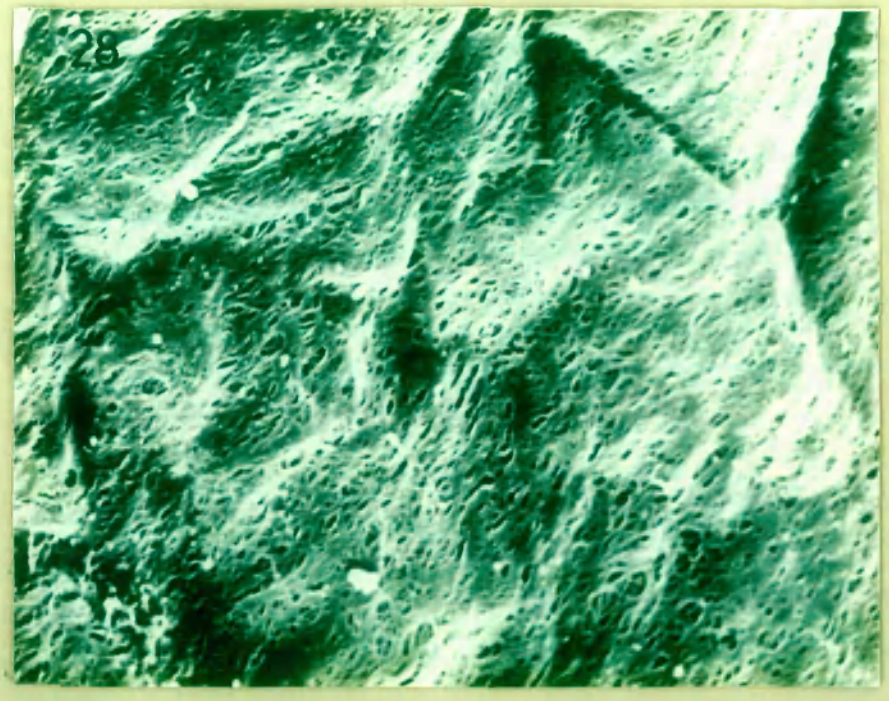
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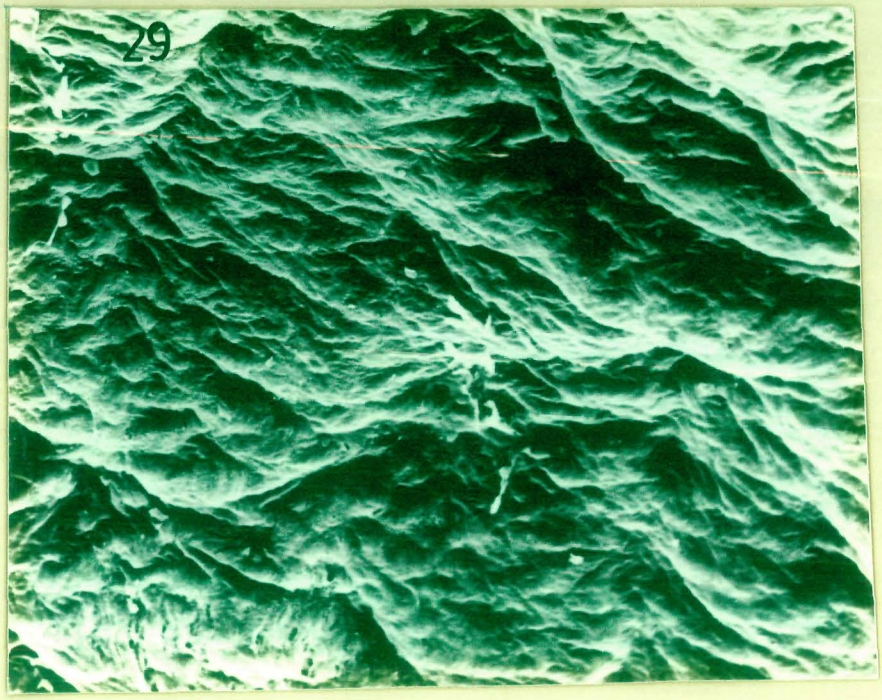
Fig.29 Scanning electron photomicrograph of the surface feature of the deeper aspect of peritoneum showing fiber bundles oriented in a regular fashion at places. Change in the direction of fibers is seen as undulating surface. Magnification. 163 x.

Fig.3Ø Surface fine structure of the peritoneum showing flat (squamous) cells and a number of pores. The undulating surface is seen as region of differential electron density - some areas are scattering more electron than others. Scanning electron photomicrograph. Magnification 155Ø X.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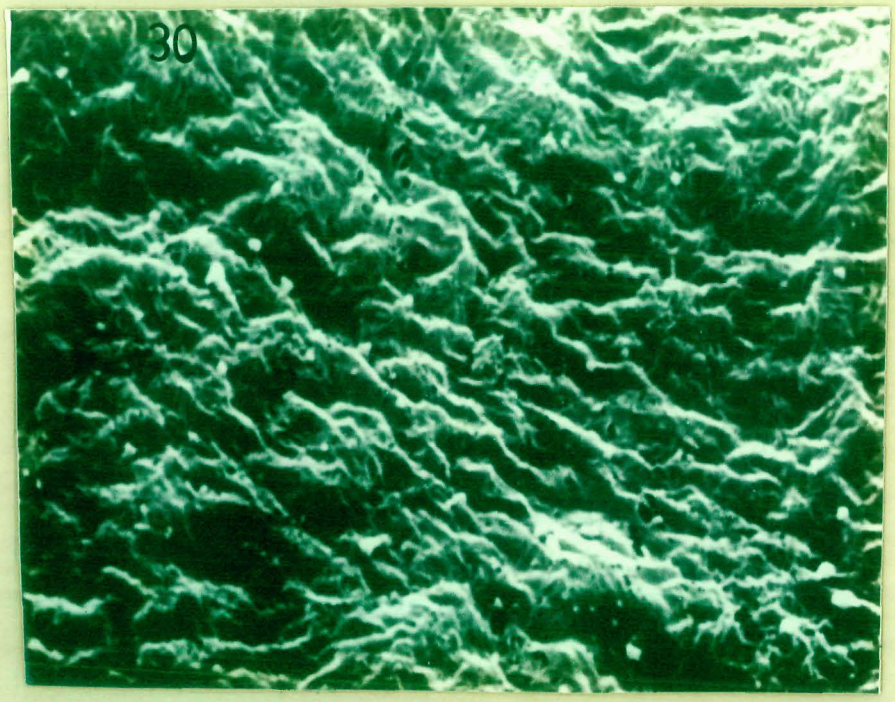
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SUMMARY

In the present thesis, three distinct types of studies have been carried out on peritoneum membrane of buffalo (Bof. bubalis). The contents of the work have been further simplified by artificially splitting them into three parts to make the discussion and analysis more meaningful.

In part one, the biophysical studies have been described. The different thermodynamic parameters such as, transport number of ions, effective fixed charge density, and permselectivity of peritoneum membrane in contact with various 1:1, 2:1 and 3:1 electrolytes have been determined from the membrane potential measurements by utilizing the widely accepted and generally used methods of Teorell-Meyer and Sievers (TMS), and most recently developed methods of Kobatake and co-workers and Tasaka et al. based on the thermodynamics of irreversible processes.

The values of membrane potential measured across peritoneum membrane with the use of NaCl, KCl, NH_4Cl, NaF, KF, KNO_3, $CaCl_2$, $MgCl_2$, $ZnCl_2$, $MnCl_2$, $CoCl_2$, $CuCl_2$, Na_2SO_4 and $CrCl_3$ have all been observed negative when the peritoneum membrane was used to separate electrolyte solution (dilute solution side was taken as negative),

meaning there by that peritoneum membrane is anion selective and positively charged. Another interesting phenomenon observed has been that when the peritoneum membrane used to separate dilute solutions, the values of membrane potential (Em or $\Delta\emptyset$) were higher and when it was separated with concentrated solution, the values were smaller. The variation of membrane potential with different electrolyte concentration may be attributed to the change in selectivity character of the membrane for ions of electrolyte at different concentrations.

For the evaluation of effective fixed charge density, Teorell-Meyer and Sievers derived the theoretical equation for the membrane potential when the charged membrane separate different concentrations of an electrolyte by considering a diffusion potential within the membrane and two interfacial potentials at the membrane solution interfaces. The membrane potential equation has been widely used for the evaluation of effective fixed charge density and mobility ratio of ions within the membrane by plotting method. The method has yielded quite satisfactory results.

Kobatake and others derived an equation for membrane potential based on the fixed charge concept by utilizing a number of basic assumptions. Two limiting forms of equation

were derived and used for the evaluation of fixed charge density of the membrane. It is interesting to note that the theoretical predictions are borne out quite satisfactorily by our experimental results with peritoneum membrane.

The equation representing the degree of permselectivity of membrane electrolyte system derived by the use of empirical expressions of the activity coefficient and mobilities of small ions in the charged membranes have been applied to peritoneum membrane. Based on permselectivity, a simple method for the determination of the effective fixed charge density, has been applied to peritoneum membrane. The permselectivity and the charge density of peritoneum membrane have been determined and the applicability of the Kobatake's equation to this system have been tested and confirmed.

Most recently Tasaka et al. derived another equation for membrane potential when a charged membrane separates two solutions of an electrolyte based on the principle of irreversible thermodynamics. At sufficiently high electrolyte concentrations, the equation reduces to a suitable form which was used for the evaluation of effective fixed charged density of the peritoneum membrane by plotting method.

The charge density values of the peritoneum membrane have been found to be of the order of 10^{-2} mole/dm^3 of the imbibed solution. The values derived from different theories are found to be almost the same and are low. The other parameters, namely t_{app}, α, β and Ps have also been evaluated. It is concluded, therfore that the different methods developed for evaluation of fixed charged density of synthetic membranes can be applied even to biological membranes.

In part second, the characterization of biochemical components from buffalo peritoneum are described. The water content was found as 57% in weight of the fresh peritoneum tissue. The protein and lipid components of peritoneum tissue were also extracted by the methods of Lowry et al. and Folch et al., respectively. The protein content was found to be 22% in weight of dry tissue while lipid is 77% of the peritoneum tissue dry weight. The compositions of total lipid fractions were resolved. Free fatty acid was found as the main component among the lipid fractions present in the peritoneum tissue fragments. The different phopholipid classes have been identified by two dimensional thin layer chromatography. Five type of phospholipids have been quantitated. Phosphatidylethanolamine and

phosphatidylcholine are the major phospholipds present in the peritoneum tissue fragments. The other phospholipds are also present namely, phosphatidylserine, phosphatidylinositol and sphingomyelin. The three ATPase system have been found in the peritoneum tissue suspension. The Mg^{+2}-ATPase activity have been found as significantly higher, among the all three ATPases studied next to follow is (Ca^{+2}-Mg^{+2})- ATPase activity. The (Na^{+}- K^{+})- ATPase activity have been observed least one among the ATPases studied in the peritoneum membrane tissue suspension. Estimation of eleven different trace elements have also been performed by atomic absorption spectrophotometry in buffalo peritoneum. Calcium, magnesium, zinc and iron are present in high abundant quantity (about 80%) in the peritoneum membrane tissue. Iron has been found as 28% of the total elements estimated in the tissue of peritoneum. The other trace metal elements which were found in peritoneum are lead, cobalt, nickel, manganese, copper, cadmium and chromium.

In the last part, the histological examination of buffalo peritoneum membrane has been done under light microscope and the surface study of peritoneum membrane has been carried under scanning electron microscope. The light

microscopic photomicrograph of the specimens show the presence of epithelial tissues, collagen fibres, blood vessels, fibroblast cells and areolar tissue. The surface view of the peritoneum membrane show the presence of uneven epithelium, flat pavement epithelium and fibre bundles. At high magnification large number of pores have also been discernible in the photomicrograph of some specimen.

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1. Arif H., Khan H.R., Shoaib, M., Ahmad M., Hasan M., Seth T.D., and Mehdi S.Q., (1989). Transport through peritoneal membrane. I-Evaluation of thermodynamic parameters. J. Member Sci. (In press).

2. Arif H., Ahmad M., Hasan M., Shoaib M., and Khan H.R. (1989). Influence of trace metal ions on the transport across pericardial membrane: Evaluation of effective fixed charge density. Ind. J. Chem. (In press).

3. Shoaib M, Khan H.R., Arif H and Ahmad M. (1989). Transport studies through peritoneal membrane II - Evaluation of effective fixed charge density. J. Non-equilib. Thermodyn. (Communicated).

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Dear Dr Arif,
Please note Dr Lonsdale's alteration of the title of your paper.
Thank you,
T. Bos

Journal of Membrane Science, 00 (1989)
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

TRANSPORT THROUGH PERITONEAL MEMBRANE I. EVALUATION OF THERMODYNAMIC PARAMETERS

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(Received March 29, 1988; accepted in revised form February 9, 1989)

Summary

The membrane potential has been measured across a peritoneal membrane separating solutions of sodium chloride, sodium fluoride, potassium chloride, potassium fluoride, potassium nitrate and ammonium chloride. These data have been used to calculate the transference numbers of ions, charge density and permselectivity of the membrane-electrolyte system. The charge density is low. The membrane potential has been found to be negative. It decreases with an increase in the electrolyte concentration across the peritoneal membrane. The peritoneal membrane has consequently been inferred to be anion selective and positively charged.

Introduction

Various attempts [1–4] have been made to study the structure and the mode of transport processes across the biological membranes. Several models have been proposed [5–11] for understanding the behaviour of the active transport and the co-transport processes in the case of artificial as well as biological membranes, for which the relevant thermodynamic parameters and the charge densities have been evaluated. Siddiqi et al. [6–7] and Beg et al. [8–10] have studied many model membranes and evaluated the charge densities and other parameters based on the principles of irreversible thermodynamics.

In view of the relevance of such studies to biological membranes, the peritoneum, being easily available, has been taken as a test membrane. Furthermore, this membrane has been used extensively for peritoneal dialysis in treating uraemia. The transport behaviour across this membrane has therefore been

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0376-7388/89/$03.50

examined by measuring the membrane potential and evaluating the effective fixed charge density as well as the permselectivity in several electrolyte solutions.

Experimental

A peritoneal membrane removed from a freshly slaughtered wild buffalo (*Bos bubalis*) aged 18–24 months was immersed in an ice-cold Ringer solution of pH 7.4 ± 0.2 for preservation. The composition of the Ringer solution was (in g/l) NaCl 8.00, $NaHCO_3$ 1.00, NaH_2PO_4 0.05, KCl 0.20, $CaCl_2$ 0.20, glucose 1.00, $MgCl_2$ 1.00. The membrane was then washed several times with deionized water to remove traces of Ringer solution before recording the membrane potential (Fig. 1) in a thermostat maintained at 25 ± 0.1 °C in the manner reported earlier [12]. The electrolytes employed were solutions of several concentrations of analytical grade (B.D.H., India) NaCl, NaF, KF, KCl, KNO_3, and NH_4Cl in deionized water. The compartment containing the more Dilute solution was taken as negative.

Results and discussion

The membrane potentials (E_m or $\Delta\phi$) recorded in millivolts are plotted against the logarithm of the average concentration, log $(C_1 + C_2)/2$, for various uni-univalent electrolytes, as shown in Fig. 2. The membrane potential has been found to decrease, after an initial increase, with an increase in the value of log $(C_1 + C_2)/2$ in the dilute range. The fact that the potential could only be recorded when the dilute side was taken as negative suggests an anion selective nature for the membrane under investigation, unlike the cation selective be-

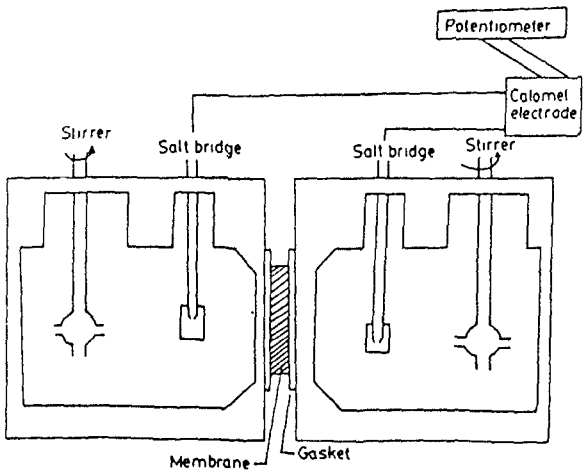

Fig. 1. Schematic diagram of cell used for the measurement of membrane potential.

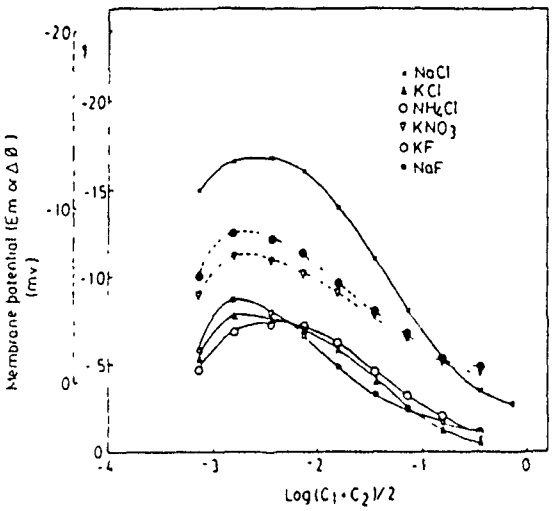

Fig. 2. Plots of observed membrane potential ($J\phi$ or E_m) against log $(C_1+C_2)2$ for various electrolytes with peritoneal membrane.

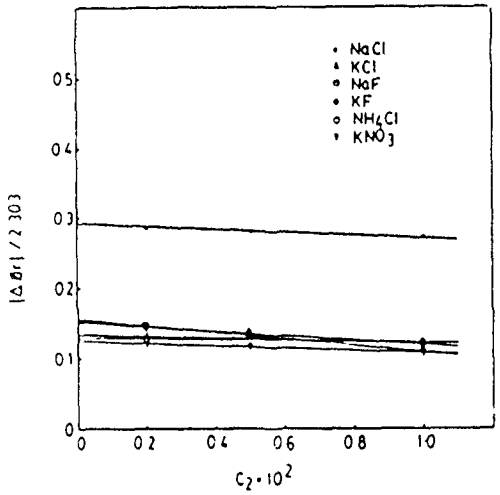

Fig. 3 Plots of $|J\phi_1/2.303$ vs. $C_2 \times 10^2$ for various electrolytes with peritoneal membrane.

haviour shown by synthetic membranes [13–18]. It may also be noted, however, that for all the electrolytes below 5×10^{-4} M concentration the number of ions generated matches the number of ions transferred to the other side of the membrane, thereby destroying completely the potential difference generated initially due to slight differences in the concentration of ions. Consequently, no potential differences is observed below the cited dilute concentration.

These data have been analysed in terms of the equations of Kobatake et al. [13] and the relevant empirical parameters, α, β and θ, computed. A variation

in the values of reduced membrane potential ($\Delta\phi_r$) with concentration, C_2 of various uni-univalent electrolytes (Fig. 3) is employed to evaluate β from the intercepts of such plots, while α is obtained from the intercept of the plot (Fig. 4). of $1/t_{app}$ (values of which are given in Table 1) versus $1/C_2$ at a fixed value of γ. These values of α and β are listed in Table 2. By employing the values of α and β, the values of θ_d are obtained. Similarly, in the concentrated range, the charge density (θ_c) has been obtained from the slope of the plots of Fig. 4 by substituting the values of α and β. These values of θ_d and θ_c along with

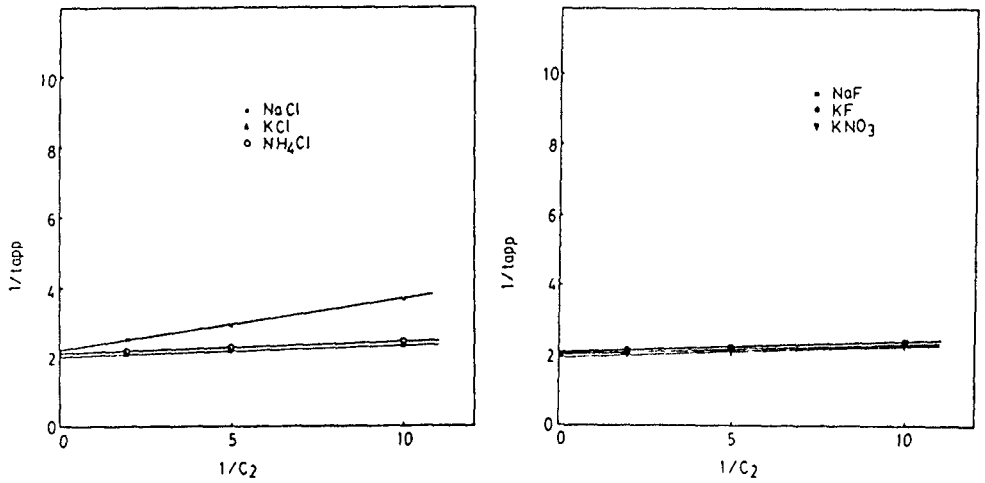

Fig 4. Plots of $1/t_{app}$ vs. $1/C_2$ for various electrolytes with peritoneal membrane

TABLE 1

The transport number (t_{app}) derived from the observed membrane potentials at various electrolyte concentrations at $\gamma = C_2/C_1 = 2$ for the peritoneal membrane

Electrolyte concentrations C_2/C_1 (mol/l)	Electrolyte					
	NaCl	KCl	NH_4Cl	KNO_3	NaF	KF
$10\times10^{-1}/5\times10^{-1}$	0.43	-	-	-	-	-
$5\times10^{-1}/2.5\times10^{-1}$	0 40	0 49	0 47	0 49	0 47	0.48
$2\times10^{-1}/1\times10^{-1}$	0 35	0 47	0 44	0 47	0.45	0 46
$1\times10^{-1}/5\times10^{-2}$	0 27	0 43	0 41	0 43	0 43	0 42
$5\times10^{-2}/2.5\times10^{-2}$	0.17	0 39	0 37	0 39	0 41	0 39
$2\times10^{-2}/1\times10^{-2}$	0.10	0 34	0 33	0 36	0 36	0 34
$1\times10^{-2}/5\times10^{-3}$	0 05	0 30	0 30	0 33	0 31	0 30
$5\times10^{-3}/2.5\times10^{-3}$	0.03	0 29	0.29	0 31	0.28	0.27
$2\times10^{-3}/1\times10^{-3}$	0.03	0 27	0 31	0 30	0 26	0.26
$1\times10^{-3}/5\times10^{-4}$	0.08	0 35	0 38	0 36	0.34	0.33

t_{app} values are obtained from eqn. (1).

TABLE 2

The derived values of parameters α and β for various electrolytes with the peritoneal membrane at $\gamma=2$

Electrolyte	α	β
NaCl	0.545	1.020
KCl	0.500	2.076
NH_4Cl	0.512	2.399
KNO_3	0.488	2.400
NaF	0.506	1.942
KF	0.500	2.007

those obtained by employing the equations of Kobatake and Kamo [17] and of Nagasawa et al. [19] and listed in Table 4, show their difference in two concentration ranges. The reason for such a difference in values seems to stem from their dependence on the electrolyte concentration [16].

Furthermore, the equation of Kobatake et al. [13]

$$\frac{\gamma-e^q}{e^q-1} = Z$$

in which

$$q=\frac{|\Delta\phi_r|+(1-2\alpha)\ln\gamma}{1/\beta+(1-2\alpha)}$$

and

$$Z=C_2/\alpha\beta\phi$$

has been found to be applicable even to biological membranes, as apparent from the plots of $\log(\gamma-e^q)/(e^q-1)$ versus $\log Z$ (Fig. 5). Also, by using the following approximate equation:

$$E_m=-\frac{RT}{F}(1-2t_{app})\ln C_2/C_1 \qquad (1)$$

the apparent transference number of the co-ions in the membrane phase, t_{app}, was evaluated. The charge density (ϕ_X) has been obtained by using the relationship:

$$\frac{1}{t_{app}}=\frac{1}{1-\alpha}+\frac{(\gamma-1)\quad\alpha}{\gamma\ln\gamma\quad(1-\alpha)}\left(\frac{\phi_X}{C_1}\right)+O\left[\left(\frac{1}{C_1}\right)^2\right] \qquad (2)$$

On neglecting the last term at higher concentrations, this equation reduces to that of a straight line. The linear plots of $1/t_{app}$ versus $1/C_1$ (Fig. 6) at a fixed

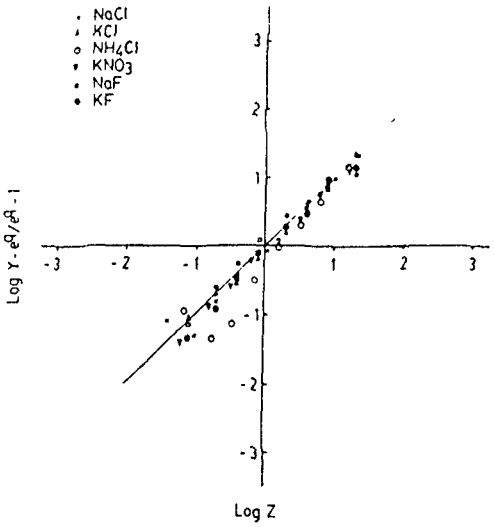

Fig. 5. Plot of log $(\gamma - e^q)/(e^q \pm 1)$ vs. log Z for various electrolytes with peritoneal membrane.

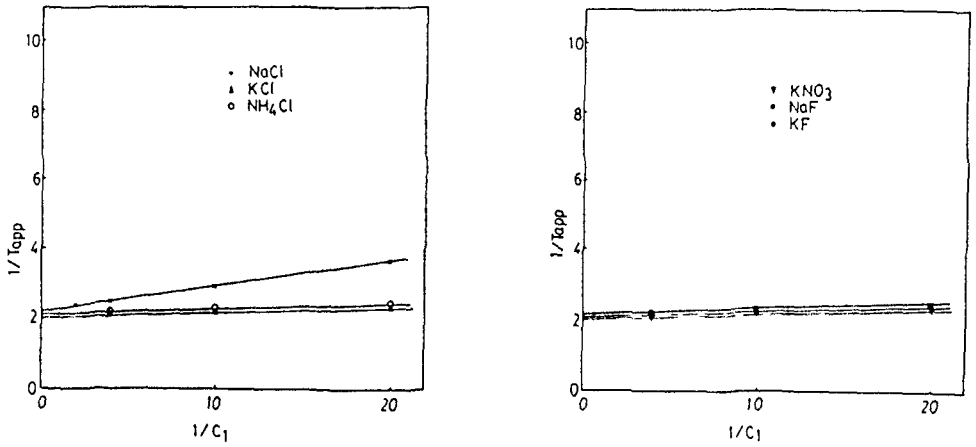

Fig. 6. Plot of $1/T_{app}$ vs. $1/C_1$ for various electrolytes with peritoneal membrane.

value of γ support the applicability of the above equation. The slopes of such plots yield the values of ϕ_X.

The values of t_{app} obtained by employing eqn. (1) have been compared with those obtained by employing the relationship:

$$T_{app} = (1-\alpha)\frac{(4\epsilon^2+1)^2+1}{(4\epsilon^2+1)+(2\alpha-1)} \quad (3)$$

As there is less than a 2% difference in the values of T_{app} and t_{app}, both are considered to be essentially the same. The values of permselectivity (P_s),

$$P_s = \frac{[T_{app} - (1-\alpha)]}{[1-\alpha-(1-2\alpha)T_{app}]} \quad (4)$$

of a positively charged membrane [16] evaluated by using values of T_{app} and α are listed in Table 3. The similarity in the pattern of plots (Fig. 7) of P_s versus log $(C_1+C_2)/2$ in the present case with those of Kobatake and Kamo [17] support the applicability of these equations even to biological membranes.

When the average concentration, $(C_1+C_2)/2$ becomes equal to the fixed charge density, the values of ϵ becomes unity, i.e., $\epsilon = C/\phi_X = 1$. On substituting

TABLE 3

The values of permselectivity (P_s) of membrane electrolyte systems for various electrolytes at different concentrations

Electrolytes concentrations C_2/C_1 (mol/l)	Electrolyte					
	NaCl	KCl	NH_4Cl	KNO_3	NaF	KF
$10\times10^{-1}/5\times10^{-1}$	0 06	-	-	-	-	-
$5\times10^{-1}/2\,5\times10^{-1}$	0.12	0 02	0 04	0 04	0 05	0.04
$2\times10^{-1}/1\times10^{-1}$	0 22	0 06	0 09	0 08	0 09	0 08
$1\times10^{-1}/5\times10^{-2}$	0.38	0.14	0 16	0 15	0 13	0 16
$5\times10^{-2}/2\,5\times10^{-2}$	0.62	0.22	0 24	0 24	0 17	0 22
$2\times10^{-2}/1\times10^{-2}$	0.76	0 32	0 32	0 30	0 27	0 32
$1\times10^{-2}/5\times10^{-3}$	0.87	0.40	0 38	0 36	0 37	0 41
$5\times10^{-3}/2\,5\times10^{-3}$	0.93	0.42	0 40	0 40	0 43	0 46
$2\times10^{-3}/1\times10^{-3}$	0.92	0 46	0 36	0 42	0 47	0.48
$1\times10^{-3}/5\times10^{-4}$	0.41	0.30	0 22	0 30	0 31	0.34

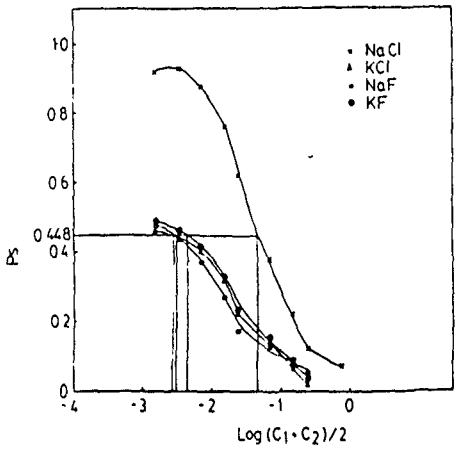

Fig. 7 Plots of P_s vs. log $(C_1+C_2)/2$ for various electrolytes with peritoneal membrane.

$\epsilon = 1$ into $P_s = (1+4\epsilon^2)^{-1/2}$, P_s turns out to have a value of 0.448. At this value, the corresponding concentration obtained from the plots of P_s vs. log C (Fig. 7) should be equal to the fixed charge density. In this way, the charge densities calculated with various electrolytes for the peritoneal membrane are given in Table 4. Using the values of ϕ_X predetermined by the method developed by Kamo et al. [16], the permselectivity of the system is re-plotted as a function of $(1+4\epsilon^2)^{-1/2}$ as shown in Fig. 8. An apparent straight line of slope close to unity is the theoretical line with the assumption that the values of ϕ_X are independent of salt concentration, as seemingly applied in the case of NaCl, but not for the other electrolytes. For these, ϕ_X seems to depend on the salt concentration, as apparent from the limiting values in plots of P_s versus $(1+4\epsilon^2)^{-1/2}$. An examination of Table 4 suggests that the extent to which the values of ϕ_X depend on the salt concentration is different in the cases of NaF, NaCl, KF, KCl, KNO_3, and NH_4Cl, for which such a dependence is quite marked. Such behaviour may consequently be understood in terms of the nature as well as the concentration of the ionic species present across the peritoneal membrane.

Tasaka et al. [19] obtained the relationship:

$$E_m = \frac{RT}{F} \ln C_2/C_1 \qquad (5)$$

TABLE 1

Charge density (mol/dm^3) of peritoneal membrane obtained by different methods at $\gamma = 2$[a]

Theory	Electrolyte					
	NaCl	KCl	NH_4Cl	KNO_3	NaF	KF
$\theta_d \times 10^2$ Kobatake's method	11.6	3.0	0.9	4.1	3.7	3.9
$\theta_c \times 10^2$ Kobatake's method	8.7	2.4	2.4	2.6	2.6	2.4
$\phi_X \times 10^2$ Kobatake's permselectivity method, P_s vs. log $(C_1+C_2)/2$	4.7	2.8	-	-	3.2	4.5
$\phi_X \times 10^2$ Kobatake and Kamo's method, eqn. (2)	8.3	1.7	1.6	2.8	1.3	1.4
$\phi_X \times 10^2$ Nagasawa's method, eqn. (6)	4.7	2.0	2.2	2.0	1.6	2.3

[a] θ_d and θ_c are obtained by using eqns. (16) and (19), respectively, of Kobatake et al. [13].

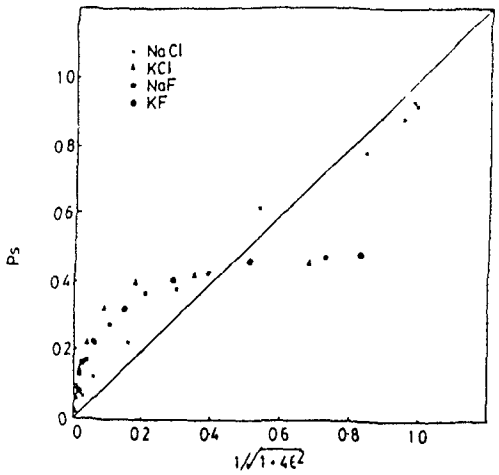

Fig. 8. Plot of P_s vs. $1/\sqrt{1+4\epsilon^2}$ for various electrolytes with peritoneal membrane.

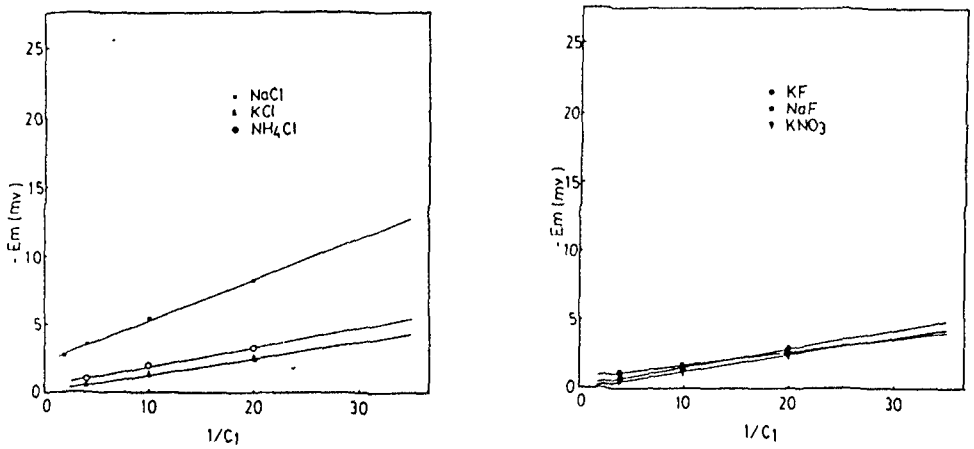

Fig. 9. Plot of membrane potential (E_m) vs. $1/C_1$ for various electrolytes with peritoneal membrane.

for extremely dilute solutions, while for higher concentrations of electrolytes the following expression:

$$E_m / \frac{\gamma - 1}{\gamma} \simeq \frac{RT\phi_X}{F} \frac{1}{2C_1} \tag{6}$$

was employed to obtain the charge density ϕ_X from the plots (Fig. 9) of E_m vs. $1/C_1$ for various 1:1 electrolytes across the peritoneal membrane. The ϕ_X values thus obtained are listed in Table 4. An examination of these plots show that the change in membrane potential is quite large with changes in concentration C_1.

Acknowledgements

The authors acknowledge with thanks the financial assistance provided by the Indian Council of Medical Research, New Delhi. They are also highly indebted to Professor Nurul Islam, Department of Chemistry, for the help rendered in the preparation of this manuscript.

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